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No. 1

UREA CLEARANCE IN NORMAL DOGS¹

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Recently Ralli, Brown and Pariente (1) and Jolliffe and Smith (2) (3) published studies dealing with "urea clearance" in the dog, similar to that carried out on man by Möller, McIntosh and Van Slyke (4) who developed this test for renal function in man.

The study of Ralli, Brown and Pariente (1) was made on dogs that excreted relatively small amounts of urine, while that of Jolliffe and Smith (2) (3) was made on dogs that excreted much larger amounts during the experimental period. Therefore, in order to determine the "augmentation limit," Jolliffe and Smith were obliged to combine their results with those of Ralli, Brown and Pariente (1).

At the time the present investigation was begun there were no publications on urea clearance in dogs. In a study on nephropathic dogs, it was considered desirable to add this test of renal function to the others used. Therefore, as a basis for comparison, the determination of urea clearance in normal dogs was first undertaken. The conditions and method of these experiments were somewhat different from those of the investigators mentioned above and the number of observations made on one series of animals much larger. Since small and large minute-volumes of urinary excretion occurred in all of the dogs, the augmentation limit could be determined on the one series of animals. For these reasons it was decided to publish these data which are very useful to us and which may prove of value to those interested in this subject.

EXPERIMENTS. Adult female dogs were used. They were of various mixed breeds, and showed no signs of renal disease as judged by the results of the examination of urine and blood. The diet consisted of about 1½ pounds of a cooked mixture of meat, bone, cabbage, oatmeal, rice and pota-

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TABLE 1
Dog 6

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DOG LENGTH	DATE	WEIGHT	SURFACE AREA (S. A.)	VOLUME OF URINE PER MINUTE (V)	\sqrt{V}	UREA NITROGEN IN URINE (U)	UREA NITROGEN IN BLOOD (B)	MAXIMUM CLEARANCE $\frac{UV}{B} = C_m$	"STANDARD" $\left\{ \frac{U\sqrt{V}}{B} = C_s \right.$ CLEARANCE"	CLEARANCE, $\frac{0.65U\sqrt{V}}{B} = C = *$	MAXIMUM CLEARANCE PER SQ. METER $\frac{UV}{B (S. A.)} = C_m / S. A.$	"STANDARD CLEARANCE" PER SQ. METER $\frac{U\sqrt{V}}{B (S. A.)} = C_s / S. A.$	CLEARANCE* PER SQ. METER $\frac{0.65U\sqrt{V}}{B (S. A.)} = C / S. A.$	AMOUNT OF WATER ADMINISTERED
cm.	1931	kgm.	sq.m.	cc.	cc.	mgm. per 100 cc.	mgm. per 100 cc.							cc.
85	4/20	18.6	0.72	0.25 0.15	0.50 0.39	1,270 1,758	9.2	34.5 28.6	69.0 73.3	44.9 47.7	47.9 39.7	95.8 101.8	62.2 66.1	
	4/21	18.4	0.71	0.07 0.06	0.27 0.24	2,014 2,150	7.7	18.3 16.8	67.8 69.8	44.1 45.4	25.8 23.7	95.5 98.3	62.0 63.9	
	4/22	18.7	0.72	0.21 0.18	0.46 0.42	1,144 1,173	9.4	25.6 22.5	55.7 53.6	36.2 34.8	35.6 31.3	77.4 74.5	50.3 48.4	
	4/25	18.0	0.71	0.08 0.06	0.28 0.24	2,055 2,137	6.5	25.3 19.7	90.4 82.2	58.7 53.4	35.6 27.7	127.2 115.7	82.7 75.2	
	4/28	18.4	0.71	0.28 0.13 0.13 0.12	0.53 0.36 0.36 0.35	1,075 2,095 2,150 2,110	9.9 8.7	30.4 27.5 32.1 29.1	57.4 76.4 89.2 83.1	37.3 49.6 58.0 54.0	42.8 38.7 45.2 41.0	80.9 107.5 125.5 117.0	52.6 69.8 81.6 76.0	184
	5/1	18.2	0.71	2.10 1.50 0.58 0.17	1.45 1.22 0.76 0.41	140 149 220 570	5.2 5.8	56.5 43.0 22.0 16.7		56.5 43.0 22.0 26.4	79.6 60.6 31.0 23.5		79.6 60.6 31.0 34.0	365
	5/7	19.4	0.73	4.87 2.38 0.38 0.18	2.21 1.54 0.62 0.42	79 112 510 799	8.2 7.2	46.9 32.5 27.1 20.1		46.9 32.5 28.4 31.1	64.3 44.5 37.1 27.5		64.3 44.5 38.9 42.6	485
	5/19	20.2	0.74	3.30 3.07 3.25 4.06 1.63 0.49	1.82 1.75 1.80 2.02 1.28 0.70	75 65 65 79 79 219	5.3 5.0 4.1	46.7 37.7 42.3 64.1 31.4 26.2		46.7 37.7 42.3 64.1 31.4 26.2	63.1 51.0 57.2 86.6 42.4 35.4		63.1 51.0 57.2 86.6 42.4 35.4	505
	12/19	23.0	0.78	0.25 0.24	0.50 0.49	3,540 4,070	33.4	26.5 29.3	53.0 59.8	34.4 38.9	34.0 37.6	67.9 76.7	44.1 49.9	

toes. On Sundays, dog biscuit was the only food given. Three times a week, half a pound of fresh, lean, ground beef was given, in addition, to every dog. Most of the animals gained some weight during the period of observation (see table 1, column 3). A standardized method was adopted for the conduct of the test.

Method of test. At 6 p.m. on the night before the test all food was removed but the animal was allowed to have water until just before the beginning of the test in the morning. Water was then removed and the dog was not permitted to drink during the test period. The test was carried out without the aid of anesthesia or narcotics and no difficulty was experienced in carrying out any of the necessary procedures. The bladder was first emptied by catheterization and this procedure was repeated at intervals of one hour for two or more hours. Time was counted from the moment the first catheterization was completed and subsequent catheterization was always begun a sufficient time before the end of each hour to insure an empty bladder at the right moment. Between 5 and 10 minutes before the catheterization for the first hour specimen of urine, blood was withdrawn by syringe from the external saphenous vein. Whenever the test was continued for four or six hours, samples of blood were again withdrawn at the end of the third and fifth hours. Four hour and six hour tests were carried out only when water was administered by stomach tube at the beginning of the experiment in order to increase the output of urine. When this was done, the water was always administered one-half hour before the first emptying of the bladder. The output of urine per minute ("V," column 5, table 1) was calculated from the measured output per hour. The urea nitrogen in the blood and urine was determined by the Van Slyke-Cullen method (5).

Urea clearance was calculated separately for every hour of the test. The determination of urea nitrogen in the one sample of blood taken in every two-hour period (column 8, table 1) served for the calculation of urea clearances for the two successive hours. Maximum clearance (C_m) (column 9, table 1) and so-called standard clearance (C_s) (column 10, table 1) with and without correction for surface area were calculated. Since high and

* Under the heading of "Clearance" (C) in column 11 are included the values of maximum clearance (C_m) for volumes of urinary excretion (V, column 5) above 0.42 cc. per minute, and the values of a standard clearance calculated by the formula $\frac{U\sqrt{V_s V}}{B (S. A.)}$ for all volumes of urinary excretion up to and including 0.42 cc. per minute, where V_s , the standard volume of reference, is taken at the augmentation limit, namely, 0.42 cc. per minute. For $V_s = 0.42$ cc., $\sqrt{V_s} = 0.65$ cc., and therefore the formula for "C" becomes $\frac{0.65 U\sqrt{V}}{B}$. In column 14 the "Clearances" per square meter $\frac{C}{S.A.}$ are listed.

low rates of urinary flow (V), (column 5, table 1) were obtained in the same dogs our data afforded an opportunity to determine the augmentation limit in one series of animals. For dogs 7, 12, 14, 17, 18 and 22, it was not possible to correct for surface area because the length of the animals had not been determined. However, their maximum and standard clearances, without correction for surface area, afforded more data for the determination of the augmentation limit, the value of which is not affected by this correc-

TABLE 2

DOG NO.	NUMBER OF OBSERVA- TIONS	MEAN C_m	MEAN C_s	MEAN C	MEAN $\frac{C_m}{S. A.}$	MEAN $\frac{C_s}{S. A.}$	MEAN $\frac{C}{S. A.}$
1	22	46.3	60.8	42.9	64.6	88.6	61.1
2	26	34.4	46.4	31.8	47.6	63.9	43.9
3	24	36.2	48.1	33.3	49.6	66.6	46.1
4	20	34.2	47.7	32.8	42.4	60.7	40.5
5	22	35.7	46.3	32.9	44.6	57.1	40.8
6	28	40.8	65.4	41.8	55.9	90.8	57.7
8	10	42.0	46.7	33.9	71.2	79.0	57.3
9	10	25.7	46.6	28.4	42.8	77.6	47.4
10	8	29.4	43.5	28.8	42.6	63.5	41.9
11	11	41.9	80.6	49.5	57.4	105.6	65.6
13	10	20.1	48.2	29.1	37.3	81.5	49.8
15	8	34.0	50.5	30.0	51.6	77.0	50.8
16	10	39.4	55.8	37.2	64.7	91.0	60.3
21	6	55.4	113.4	64.6	76.3	159.9	90.1
25	6	33.8	55.8	35.4	56.3	94.1	59.5
39	8	41.9	75.8	46.6	63.5	113.9	70.1
40	5	48.4	56.1	38.8	73.4	108.4	71.1
7	8	26.7	31.1	23.4			
12	10	22.9	45.4	26.2			
14	8	31.2	52.8	33.2			
17	8	39.9	65.5	41.6			
18	10	41.0	55.1	36.8			
22	6	35.2	51.9	33.9			
Mean values for all the animals		36.6 \pm 8.1	55.0 \pm 12.4	36.0 \pm 8.3	53.1 \pm 11.4	80.9 \pm 18.7	52.7 \pm 12.6

tion, as used in this study. The augmentation limit, therefore, was determined from the average values of C_m and C_s for all the dogs.

On account of lack of space, the complete data for only one dog are given, as an illustration, in table 1. Table 2 is a summary in which are given the mean values of the determinations for individual dogs and the mean values for the 23 dogs.

The mean value of C_m , for values of V above 0.42 cc. per minute for the 23 dogs (111 observations) was 36.6 cc. \pm 8.1.

The mean value of C_s , for values of V up to and including 0.42 cc. per minute, for the 23 dogs (173 observations) was 55.0 cc. ± 12.4 .

From these two mean values the volume of the augmentation limit can be calculated, and was found to be 0.43 cc. For 17 of the 23 dogs studied the values of C_m and C_s per square meter of surface area $\frac{(C_m)}{(SA)}$ and $\frac{(C_s)}{(SA)}$ were calculated. This made the clearances in different dogs more comparable since the animals varied considerably in weight and length. Surface area (S.A.) was determined by the following formula of Cowgill and Drabkin (6).

$$S.A. = 4.381 W^{0.425} \times L^{0.725}$$

S.A. = Surface area in square meters.

W. = Weight in grams.

L. = Length in centimeters.

(In table 1, W and L appear as kilograms and meters respectively.)

The mean value of $\frac{C_m}{S.A.}$ (93 observations) was 53.1 cc. ± 11.4 .

The mean value of $\frac{C_s}{S.A.}$ (141 observations) was 80.9 cc. ± 18.7 .

The augmentation limit was 0.42 cc.

The augmentation limit for the smaller number of dogs was almost the same as the value obtained for all of the dogs, without correction for surface area.

Chart 1 illustrates graphically the values of $\frac{C_m}{(S.A.)}$ for the 17 dogs of known surface area and gives the position of the augmentation limit.

In order to be able to compare directly the values for maximum and standard clearances, previous investigators have calculated the percentage value of all of the observations, taking the mean C_m and mean C_s each as 100 per cent. In this study, to accomplish the same purpose, a method of calculation was used suggested to us by Dr. R. Dominguez, Director of Laboratories, St. Luke's Hospital, Cleveland. This method consists in the calculation of a standard clearance for all minute volumes of urine below the augmentation limit, by using the value of the augmentation limit itself, namely, 0.42 cc. per minute, instead of 1 cc. per minute, as the standard volume of reference.

Möller, McIntosh and Van Slyke (4) have shown that

$$C_s = C_m \cdot \sqrt{\frac{V_s}{V}},$$

where V_s is the standard volume of reference, that is,

$$C_s = \frac{UV}{B} \cdot \sqrt{\frac{V_s}{V}} = U \frac{\sqrt{V \cdot V_s}}{B}$$

and when $V_s = 0.42$ cc. (the augmentation limit), then $\sqrt{V_s} = 0.65$ and $C_s = \frac{0.65 U \sqrt{V}}{B}$, or $\frac{C_s}{S.A.} = \frac{0.65 U \sqrt{V}}{B (S.A.)}$. This method was mentioned but not favored by Möller (7), in the case of human beings, because he considered the variation of the individual augmentation limits too great to justify this procedure. In dogs, the variation of individual augmentation limits is also great. However, the choice of this value as the standard volume of reference, in addition to making standard and maximum clearances directly comparable is further justified by the fact that this volume (0.42 cc. per minute) is a natural standard for the dog, since it is close to the average rate of urinary excretion per minute of the adult animal. In

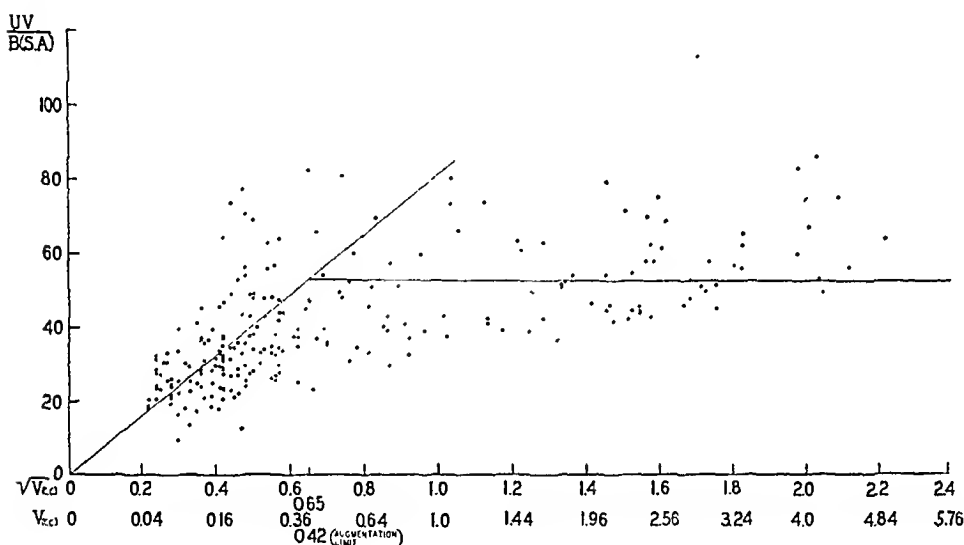


Chart 1

the 23 dogs (44 determinations) the range of excretion in 24 hours was 150 cc. to 1080 cc., with a mean value of 489 cc., which is 0.34 cc. per minute.

With all of the standard clearance values referred to the augmentation limit volume as the standard volume of reference, maximum and standard clearance values can be put in the same column and the values can then be termed collectively the *CLEARANCE* (C) (table 1, column 11). The mean value of (C) for the 23 dogs (284 observations) was $36.0 \text{ cc.} \pm 8.3$, which is practically the same as the value $36.6 \text{ cc.} \pm 8.2$ of C_m . The mean value of $\frac{C}{(S.A.)}$ for 17 dogs of known surface area, (234 observations) was 52.7 cc.

± 12.6 , per square meter, which is practically the same as that of $\frac{C_m}{S.A.}$, $53.1 \text{ cc.} \pm 11.4$. The close correspondence of these values is to be expected

from the nature of the calculations, if the augmentation limit is a good choice as the standard volume of reference.

SUMMARY

In 23 normal dogs urea clearance and augmentation limit were determined. The mean value of the so-called standard clearances, with 1 cc. per minute taken as the standard volume of reference, and uncorrected for surface area, was 55.0 cc. \pm 12.4 and, corrected for surface area, 80.9 cc. \pm 18.7 per square meter of body surface. The mean value of the maximum clearances, uncorrected for surface area, was 36.6 cc. \pm 8.1 and, corrected for surface area, 53.1 cc. \pm 11.4 per square meter. The "augmentation limit," as determined by the urea clearances of 23 dogs (284 observations) uncorrected for surface area, was 0.43 cc. When determined by the urea clearances of only 17 dogs (234 observations), corrected for surface area, the augmentation limit was 0.42 cc. It is suggested that for the purpose of direct comparison of urea clearances at all values of V (column 5, table 1), and to avoid the so-called standard clearance, the value of the augmentation limit (0.42 cc.) should be used as the standard volume of reference, for a value is thus obtained which is truly a clearance. This makes it possible to include all values under the one term "urea clearance" without reference to "standard" and maximum clearances. In 23 dogs the mean value of the "urea clearance" (C) calculated according to this method was 36.0 cc. \pm 8.3. In 17 dogs, of known surface area, $\frac{C}{(S.A.)}$ was 52.7 cc. \pm 12.6 per square meter. These values are practically identical with the mean values of the maximum clearances (C_m) 36.6 cc. \pm 8.1 and $\frac{C_m}{(S.A.)}$ 53.1 cc. \pm 11.4 per square meter, respectively, as is to be expected when the augmentation limit is used as the standard volume of reference.

BIBLIOGRAPHY

- (1) RALLI, E. P., M. BROWN AND A. PARIENTE. *This Journal*, 1931, xcvii, 432.
- (2) JOLLIFFE, N. AND H. W. SMITH. *This Journal*, 1931, xcvi, 572.
- (3) JOLLIFFE, N. AND H. W. SMITH. *This Journal*, 1931, xcix, 101.
- (4) MÖLLER, E., J. F. MCINTOSH AND D. D. VAN SLYKE. *Journ. Clin. Invest.*, 1928, vi, 367.
- (5) VAN SLYKE, D. D. AND G. E. CULLEN. *Journ. Biol. Chem.*, 1914, xix, 211.
- (6) COWGILL, G. R. AND D. L. DRABKIN. *This Journal*, 1927, lxxxi, 36.
- (7) MÖLLER, E. *Acta. Med. Scand.*, 1927, Supplement 26, 259.

AN EXPERIMENTAL STUDY OF "THE CONSTITUTIONAL FACTOR" IN THE ETIOLOGY OF RICKETS

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It has long been felt that, in addition to the hygienic and dietetic factors which undoubtedly play the important rôle in the etiology of rickets, some undefined and more subtle factor is of significance in the development of this disorder. For want of a more precise designation, this generally has been termed the constitutional factor. At various times, one of us has commented on this vague but nevertheless definite causative influence. In 1928, it was observed that "differences in the percentage of ash in the skeleton at birth and in the rate of growth in the early months of post-natal life are undoubtedly of importance, but in all probability deeper biologic influences of which we have no knowledge also play a rôle" (1). Somewhat later the subject was referred to in the following words: "There is some important factor in connection with the development of rickets which we have not yet fathomed and we have not yet taken sufficiently into account. Some have termed this the constitutional factor" (2). In regard to the negro infant, which is notably more susceptible to rickets than the white infant, it was felt that "in addition to this well recognized factor (pigment of the skin) the negro may possess an inherent racial tendency to rickets—a tendency perhaps shared by other races" (3). Although the reality of a constitutional disposition has been recognized, the subject has never been approached or studied from the experimental standpoint.

Recently the opportunity was presented of demonstrating differences in the development of rickets among individuals of a litter of puppies which had been reared under the same conditions in regard to diet and general surroundings. These four puppies were of mongrel breed, two being females and two males. It may be noted from the accompanying photographs that two were predominantly of one type, a short-haired breed, whereas the other two were of a long-haired terrier type. This distinction was evident at a glance, although there was a gradual gradation from one type to the other. Immediately after weaning, these four puppies were kept in separate cages in one room and fed the Mellanby rickets-producing diet, which consists of skim milk, cereal, cottonseed oil, sodium chloride and

sufficient tomato juice daily to prevent scurvy; it is a low calcium, high phosphorus ration. The control animal (A) which has a normal appearance both in the photograph and radiograph, received also a supplement of viosterol. The feeding experiment was carried out from November to March, a period of almost five months. The accompanying photographs show that the dog which received viosterol appeared normal with strong

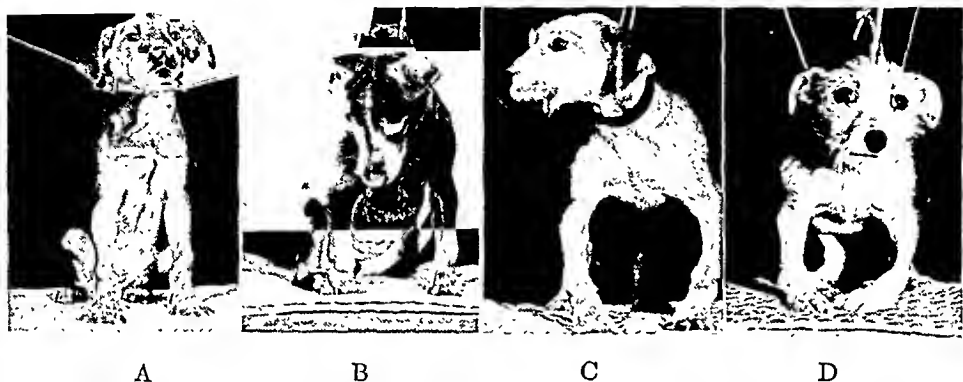


Fig. 1. Four mongrel puppies of the same litter, ranging from short-haired to long-haired type. Development and intensity of rachitic lesions at the epiphyses and bowing according to preponderance of long-haired type.

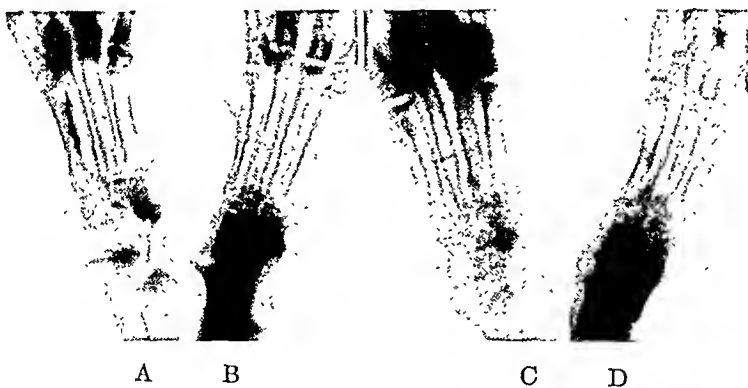


Fig. 2. Radiographs of the puppies in preceding figure; showing same individual distribution and intensity of rickets. Note increasing width of carpal and metacarpal junctions.

straight legs, that the other short haired puppy (B) had mild rickets as evidenced by bowing of the legs and enlargement of the epiphyses at the ankle joints, that the third, somewhat shaggy puppy (C), had marked bowing of the legs, and that the legs of the fourth animal (D) were so bowed as to prevent her from standing unassisted. The radiographs of the ankles of the forelegs corroborated these appearances, showing normal

joints in the first puppy, moderate rickets in the two succeeding puppies, and marked rickets in the fourth animal.

The accompanying table gives the concentration of the calcium and inorganic phosphorus of the serum at the mid-period and at the end of the experiment. It will be noted that, whereas the serum was normal in the animal which received viosterol, it showed a definite and marked decrease of calcium in the three other animals; in the puppy with the most marked bowing of the legs, there was not only the lowest concentration of calcium, but likewise the lowest percentage of inorganic phosphorus.

As is well known, growth is an important factor in the development of rickets, the greater the growth the more marked the tendency to the development of rickets in infants and in animals. In fact, lack of growth works strongly against the induction of this disorder. In the accompanying table it may be noted that the puppy which developed rickets in great-

TABLE 1
Concentration of calcium and of inorganic phosphorus in serum of the four puppies

PUPPY	WEIGHT		SERUM CALCIUM		SERUM PHOSPHORUS	
	At onset	At end	Mid-period	At end	Mid-period	At end
	<i>kgm.</i>	<i>kgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
A ♂	2.3	6.0	10.0	11.1	8.2	8.3
B ♀	1.85	5.7	7.1	6.1	6.3	5.8
C ♂	2.9	5.84	6.6	7.0	6.6	5.8
D ♀	2.14	3.71	5.7	5.5	6.0	5.6

est intensity made the least gain during the three months period, so that growth can be excluded as an inciting factor in this experiment.

A result such as the foregoing seems to indicate that one breed of dog is more susceptible to rickets than another. In other words it can be demonstrated that there is a definite constitutional tendency to rickets, quite apart from diet, hygiene and growth. This must be evident to those who have studied clinical rickets. Among infants brought up in the same institution and receiving the same diet and the same care, some develop normally whereas others develop moderate or even a marked degree of rickets. A similar distinction, from the point of view of constitution, holds for other disorders of the bones and is a factor in the varying susceptibility of the teeth to caries.¹ The method of approach which we have outlined seems well suited not only for studies of the constitutional factor in rickets but of other disturbances in the development of the bony skeleton.

¹ The teeth of these puppies are being subjected to histologic study by investigators of the Committee on Dental Caries, supported by the Commonwealth Fund.

BIBLIOGRAPHY

- (1) HESS, A. F. AND M. WEINSTOCK. Amer. Journ. Dis. Child., 1928, xxxvi, 966.
- (2) HESS, A. F. Acta paed., 1930, xi, 1.
- (3) HESS, A. F. Rickets, osteomalacia and tetany. 1929, Lea & Febiger, p. 89.

THE CHEMICAL MEDIATION OF AUTONOMIC NERVOUS IMPULSES AS EVIDENCED BY SUMMATION OF RESPONSES

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Recent studies have shown that the stimulation of autonomic nerves sets free chemical substances which may pass into the blood stream and show a sympathomimetic or a parasympathomimetic action on other structures (see Cannon, 1931, for bibliography). The studies on the sympathetic give support to the idea expressed by Elliott (1904), that sympathetic impulses liberate adrenin locally and that it is this adrenin which conditions the response of the effector.

It has been recently shown that the curves of the responses to varying doses of adrenin are hyperbolas and that the mode of action of the hormone is therefore probably chemical (Rosenblueth, 1932). From the all-or-none character of nervous impulses it follows that the amounts of mediator produced must be proportional to the frequency of the impulses, and, if the hypothesis of a chemical mediation be correct, the curves of the responses to varying frequencies up to a limit which will involve the refractory period of the nerve or the end organ should be identical with those obtained by varying doses of adrenin.

The present paper is a quantitative study of the responses of different structures innervated by the autonomic nervous system to varying frequencies of stimuli.

METHODS. Cats were used for all experiments except some on the submaxillary gland, in which dogs were found more suitable.

Dial anesthesia (0.6 to 0.9 cc. per kilo intraperitoneally or by stomach tube) was utilized, save in the observations on the actions of the splanchnic on the intestine and the accelerators on the heart. Since anesthetics are likely to modify the responses of these organs, the animals were swiftly pithed through the temporal bone and then used without anesthesia (see Cannon and Britton, 1925).

The curves obtained from the data furnished by the experiments were tested by the usual methods. Semi-logarithmic and logarithmic plottings eliminated exponentials and parabolas. After adjustment to adequate

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scales, symmetry of the curve to the bisector of the right angle formed by the two asymptotes was examined by transparence after folding the paper along the line. The curves were invariably found to be symmetrical to this line, as befits a hyperbola. Parabolas and exponentials would not show this symmetry. Finally the curves were studied numerically, as described in the preceding paper (Rosenblueth, 1932), i.e., the constancy of the product $(b - y)(a + x)$ was investigated, where y is the response, x represents the frequency (number of discharges per second), and a and b are constants obtained by the method of least squares. This latter test is the most significant and its results will be given in most instances.

The numerical test for the hyperbolas gave approximations usually closer than 10 per cent, a result quite satisfactory in consideration of the methods employed. The tests for other types of curves gave greater deviations, and, what is more important, the errors were definitely and consistently systematized. This did not occur when they were considered as hyperbolas.

In every case several readings were taken on each frequency. A random order was commonly followed. The results were found to coincide satisfactorily.

The stimulator used was designed and constructed by Mr. E. L. Garceau, electrical engineer, in this Laboratory. The following is Mr. Garceau's description of the apparatus:

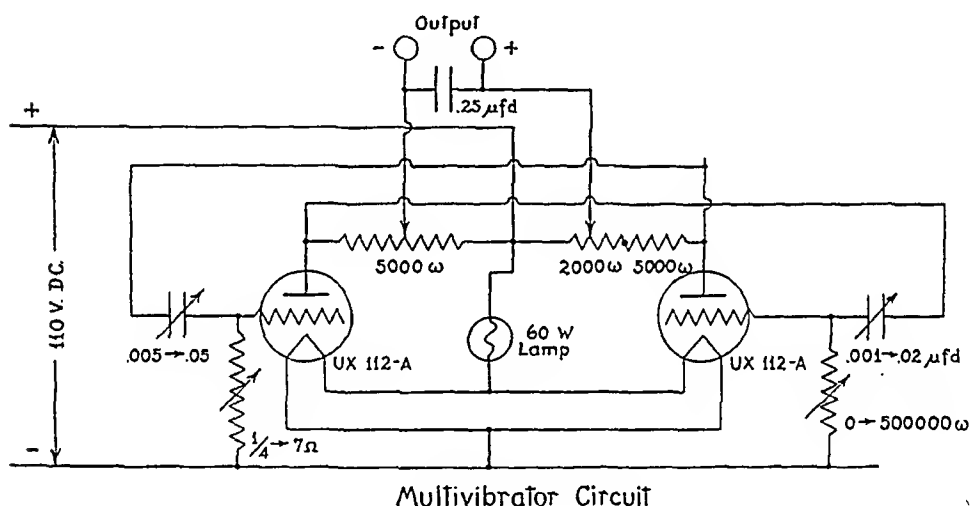
The multivibrator stimulator (see accompanying diagram) is an adaptation of the multivibrator circuit known for some time in electrical communication engineering. It consists essentially of two resistance-capacity coupled amplifier tubes (UX 112A). The plate circuit of each tube feeds into the grid circuit of the other.

In the diagram imagine a minute negative potential from any cause to be impressed upon the grid of one tube. The plate of the tube will then go slightly positive as the space current is decreased, and the grid of the second tube will also go positive. The space current of the second tube will increase, and its plate will therefore go negative, impressing a further negative potential on the first grid. This goes on very rapidly until the first grid is so far negative that the plate current of the first tube is entirely cut off. Now the grid condenser of the first tube discharges its excess charge through the grid leak. During this time there is no plate current in the first tube, but a large one in the second. When the grid condenser has discharged sufficiently to allow the first plate to take current, the grid of the second tube is falling in potential and it blocks in turn. The time of blocking of each tube is nearly proportional to the value of the product of grid leak and coupling capacity of RC. The plate resistances are potentiometers and the stimulating circuit is connected to the moving contacts of these. The circuit is unsymmetrical in that one tube is made to block much longer than the other. Both condensers and grid leaks are variable, and the stimuli are intense pulses of short duration derived from one potentiometer, separated by long intervals during which a small depolarizing voltage flows from the other potentiometer.

The wave form is closely rectangular. The number of stimuli may vary from one in several seconds to 10,000 or more per second. It is difficult to obtain a ratio of

stimulating time to depolarizing time of more than 20 to 1. Either voltage in this apparatus may be run up to about 40 volts. The device may be calibrated very accurately with a cathode ray oscillograph using a linear time axis or with any good photographic oscillograph. It is possible to vary independently the stimulator voltage, stimulating time, depolarizing voltage and time between stimuli. This form of the apparatus works directly from 110 volt direct current line. The plate voltage is derived from a line directly and the filament current is reduced by means of a 60 watt tungsten lamp. It is important to use the correct polarity of the line and to insulate the preparation from ground.

The temperature and the intensity affect the absolute frequency, although the relative scale of the frequencies is not modified. To eliminate any possible error the frequencies were usually recorded and thus determined. The figures of the conventional scale on the dial of the correspond-



ing potentiometer varied practically in a linear relation to the intensities of the stimuli, because of the low resistance of this potentiometer. On this scale, 30 was found to be approximately equal to 10 volts. The duration of each stimulus used was from 0.5 to 10 σ .

RESULTS. A. STRUCTURES INNERVATED BY THE SYMPATHETIC. 1. *Single hair of the tail of the cat.* It was deemed desirable to obtain information on the action of a neuromuscular preparation as simple as possible. A single hair in the tail of the cat was chosen because it is moved by a slender bundle of smooth muscle cells which are parallel and therefore act in one direction only.

Histological evidence was not found in the literature regarding the number of nerve fibers supplying the *arrectores pilorum*. The physiological action,

however, was always, as will be shown below, that of a single neuromuscular unit. Preganglionic fibers were usually stimulated—each fiber being distributed to a larger number of postganglionic elements. In some cases, however, the postganglionic fibers were stimulated and no differences appeared in the observations. If there be a plural postganglionic innervation of the muscle the thresholds of the fibers concerned must be very similar.

One hair was isolated by clipping short all those surrounding it. At rest it lies nearly parallel to the skin and pointing toward the tip of the tail. Stimulation of the lower abdominal sympathetic chains will make the hair describe an arc of a circle whose center is the point of its insertion in the skin.

The movement is at first rapid, then slower, and finally the hair remains at a given position as long as the stimulus is maintained. One minute is usually more than sufficient time to reach this state of equilibrium. Relaxation after the stimulus ceases shows the same, probably exponential, changes in rate, beginning rapidly and becoming continuously slower.

A protractor, held by a clamp so that its diameter was touching the skin, its center coinciding with the base of the hair, and its surface parallel to and close to the plane of movement, permitted the movements of the hair to be read directly and with an accuracy of 0.5° . Records of the movement were regarded as unnecessary since the interesting feature was the maximal excursion of the hair with the different rates of stimulation.

It was assumed that the amount of contraction developed by the muscle is approximately linearly proportional to the angle described by the hair. This assumption rests on the plausible hypothesis of a practically linear resistance offered by the elasticity of the structures opposing the movement. These structures bring the hair back to its resting position when contraction ceases.

If the nerve is stimulated for a certain period (30 to 60 seconds) with a given frequency, and the intensity is gradually increased, there is a narrow threshold zone in which there is gradation of the responses, but the maximal response for that frequency is rapidly reached. Further increases of intensity have no effect. The zone where gradation occurs can be accounted for by summation of subliminal stimuli or by fluctuations in the thresholds and in the refractory periods or, finally, by the influence of the process of depolarization. In no case was there any evidence of steps in the gradation which would imply a plural innervation of the muscle.

Following are the data of a typical observation:

Frequency = 15 shocks per second. The intensities are expressed in the conventional units of the scale of the stimulator (see description above).

INTENSITIES (IN THE ORDER IN WHICH THEY WERE APPLIED)	ANGLES DESCRIBED BY HAIR
10	0°
15	15
12	10
17	16
20	17
13	15
12	12
15	17
10	0
11	5

Single discharges from the stimulator, if long enough to satisfy the "excitation time," will also produce a response. The threshold is then very sharp and the response is maximal from the start. Here again no steps were ever observed.

Following are the data of a typical observation:

Single shocks

INTENSITIES (IN THE ORDER IN WHICH THEY WERE APPLIED)	ANGLES DESCRIBED BY HAIR
5	0°
7	9
10	8
6	0
7	8
9	9
8	9

These reactions to changes in the intensity of the stimulus indicate a simple neuromuscular mechanism, probably a single unit. Also, these results agree with the classical notion of the all-or-none response of the nerve.

The influence of varying frequencies was studied by applying supra-maximal stimuli (about twice the threshold) for a time sufficient to produce a state of equilibrium (tetanus). Here a wide-ranged gradation occurs, the height of the contraction increasing with the frequency (summation) until a frequency limit is reached beyond which a sharp initial rise is followed by a rapid decline to a lower level, which may be sustained for some time. The interpretation of this action of too frequent stimuli will be taken up in the discussion; the same phenomenon occurred in all the responses studied.

Within stimulation frequencies that produce a sustained contraction the results of a typical experiment are illustrated in figure 1-A.

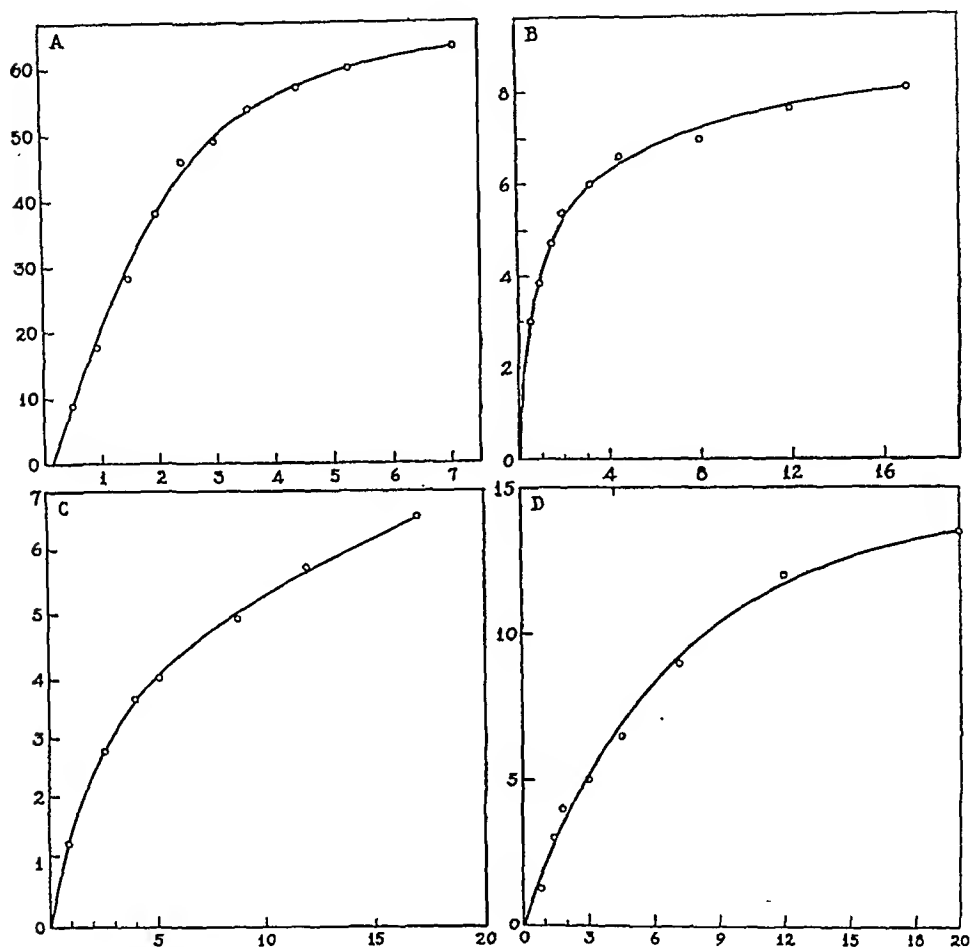


Fig. 1-A. Abscissae: frequencies (shocks per second) of stimulation of the lower abdominal sympathetic chains at L5. Ordinate: angles (in degrees) of movement of a hair in the tail of a cat, averages of several observations. See text for test of this and all other curves.

B. Curve plotted from the results obtained in the experiment illustrated in figure 2. Abscissae: frequencies of stimulation; ordinate: height of contraction in the record 15 seconds after the beginning of stimulation.

C. Curve plotted from the results obtained in the experiment illustrated in figure 3. Abscissae: frequencies of stimulation; ordinate: height of contraction in the record 15 seconds after the beginning of stimulation.

D. Abscissae: frequencies of stimulation of right heart-accelerators. Ordinate: maximal increase of the heart-rate per 15 seconds, averages of several observations.

The method described above to prove that it is a hyperbola yields the following figures:

$$a = 1; b = 77.7$$

x	y	$(b - y) (a + x)$
0.5	9	103
0.9	20	110
1.5	28	124
2.0	38	119
2.4	46	108
3.0	49	115
3.6	54	109
4.4	57	112
5.3	60	112
7.1	63	119
Average.....		113

Maximal deviations +11; -10.

2. *Nictitating membrane of the cat.* The distal end of the cut cervical sympathetic was stimulated. Isotonic and isometric contractions were recorded as described by Rosenblueth (1932). An intensity was chosen higher than that necessary to produce a maximal response at a given frequency. This intensity was now applied at varying frequencies. The results are illustrated in figures 1-B and C, 2 and 3. Analysis of the curves gives the following values:

Figure 1-B

$$a = 2; b = 8.75$$

x	y	$(b - y) (a + x)$
0.5	3.0	14.5
0.9	3.9	14.2
1.4	4.7	13.9
2.0	5.4	13.6
3.2	6.0	14.5
4.6	6.6	14.5
8.0	7.1	16.3
12.0	7.6	16.1
17.0	8.1	14.2
Average.....		14.6

Maximal deviations +1.7; -1.

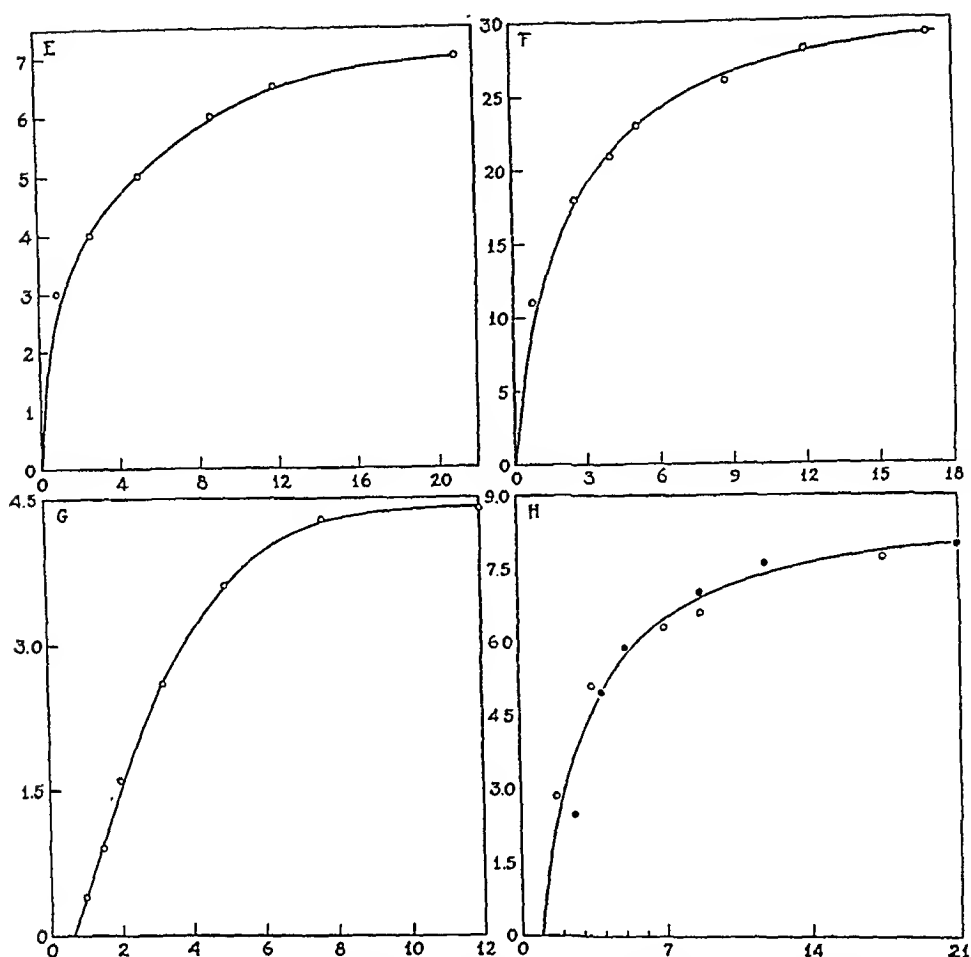


Fig. 1-E. Curve plotted from the results obtained in the experiment illustrated in figure 4. Abscissae: frequencies of stimulation; ordinates: average heights of the contractions in the record.

F. Adrenals removed, right splanchnics cut. Abscissae: frequencies of stimulation of the left splanchnics. Ordinates: average heights of inhibition of the duodenum in the record. See text for explanation of method used.

G. Curve plotted from the results obtained in the experiment illustrated in figure 5. Abscissae: frequencies of stimulation of the adrenal; ordinates: average maximal rises of blood pressure in the record.

H. Abscissae: frequencies of stimulation of the left splanchnics for one minute (dots) and doses of adrenalin injected in one minute (circles), unit of adrenalin 0.000286 mgm. Ordinates: maximal height of contraction of the nictitating membrane in the record. Magnification, 11.

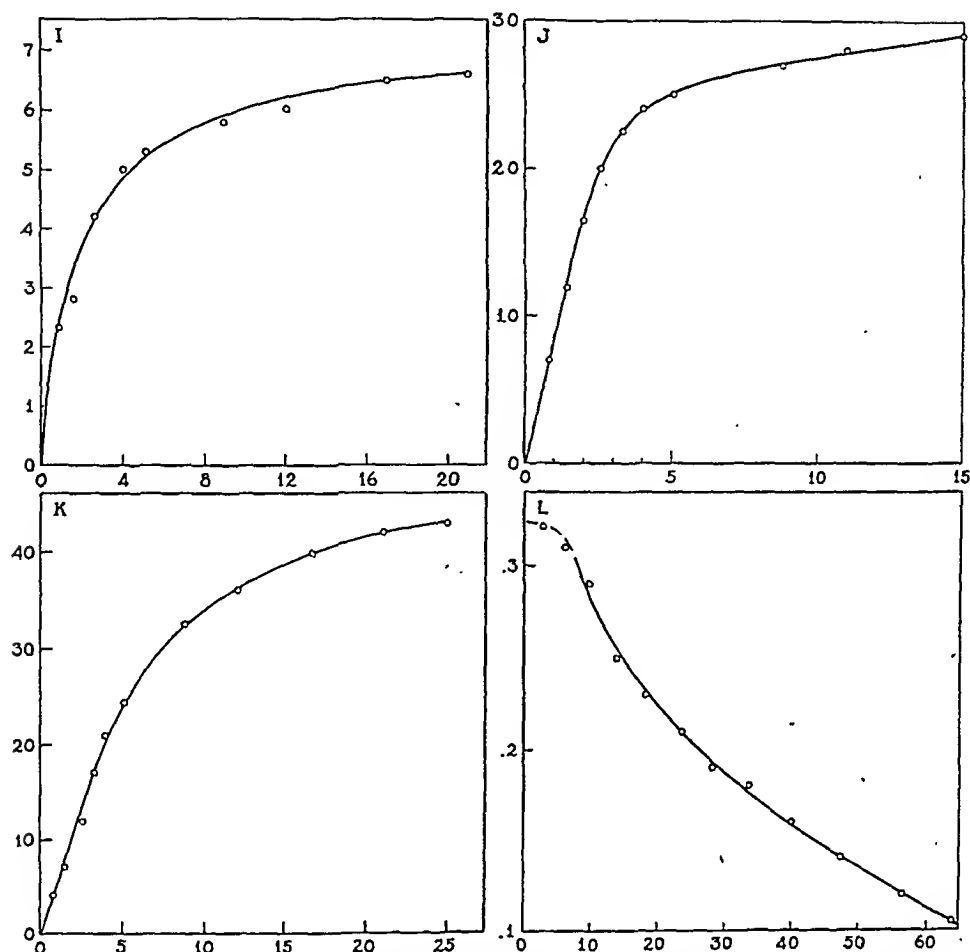


Fig. 1-I. Curve plotted from the results obtained in the experiment illustrated in figure 6. Abscissae: frequencies of stimulation; ordinates: mean height of the contractions in the record.

J. Abscissae: frequencies of stimulation of the right vagus. Ordinates: maximal slowing of the heart-rate per 15 seconds.

K. Curve plotted from the results obtained in the experiment illustrated in figure 7 and from several other observations on the same animal. Abscissae: frequencies of stimulation; ordinates: average total number of drops of saliva secreted.

L. Abscissae: time in millimeters on the record. Ordinates: rate of flow of saliva measured by taking the reciprocal of the intervals between the drops in millimeters.

Figure 1-C
 $a = 14.4; b = 10.4$

x	y	$(b - y) (a + x)$
0.9	1.3	139
2.6	2.7	131
4.0	3.5	127
5.1	3.8	129
8.8	4.7	132
12.0	5.5	129
17.0	6.3	129
Average.....		131

Maximal deviations +8; -4.

These are, therefore, again hyperbolas.

Figures 2 and 3 present two features which were consistently found in other experiments. The level of maximal contraction (plateau) is at first horizontal; after a given critical frequency there occurs the usual exponential rise followed by a continuous slow linear ascension, so that the "plateaus" are no longer horizontal. The slope of this slow ascension is steeper as the frequency increases. The second characteristic to be observed in these curves is the longer after-effect as the frequency increases. The explanation of these facts will be taken up in the discussion.

3. *Accelerator of the heart.* The vagi were cut in the neck, after insertion of a tracheal cannula for spontaneous and artificial respiration. The stellate ganglia were approached through the right third intercostal space. The left one was removed. The superior, inferior and external branches of the right one were cut and shielded electrodes were applied to the internal branches. Supramaximal stimuli were applied with varying frequencies.

The results of a typical instance are illustrated in figure 1-D. The usual test for a hyperbola is the following:

$$a = 7.4; b = 18.6$$

x	y	$(b - y) (a + x)$
0.8	1.5	140.2
1.4	3.0	137.3
1.8	4.0	134.3
3.0	5.0	141.4
4.6	6.5	145.2
7.2	9.0	140.2
12.0	12.0	128.0
20.0	13.5	139.7
Average.....		138.3

Maximal deviations +6.9; -10.3.

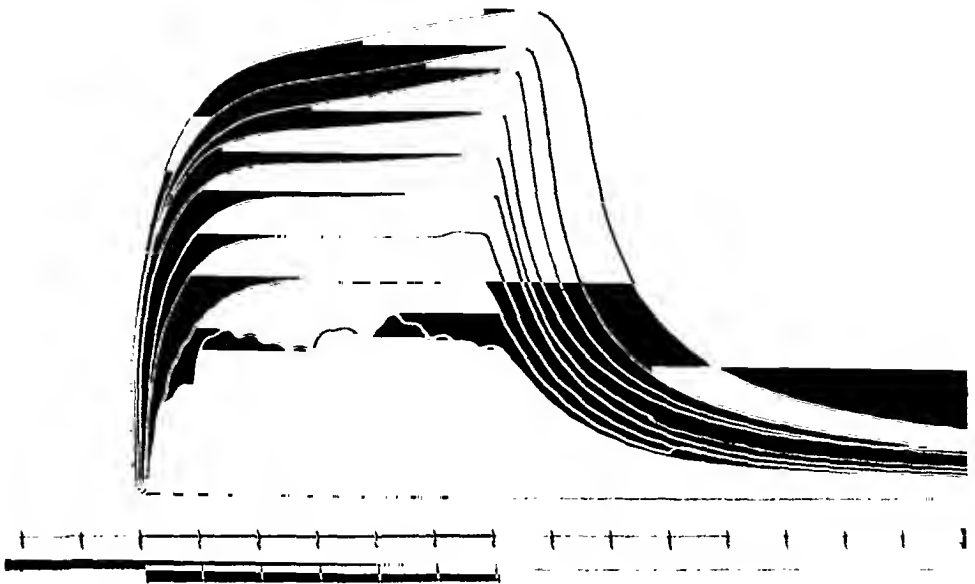


Fig. 2. Isotonic contractions of the nictitating membrane on stimulation of the cervical sympathetic with the following frequencies: 0.5, 0.9, 1.4, 2, 3.2, 4.6, 8, 12 and 17 supramaximal shocks per second. Time recorded in 5 second intervals. Magnification, 12. Tension on muscle, 4 grams.

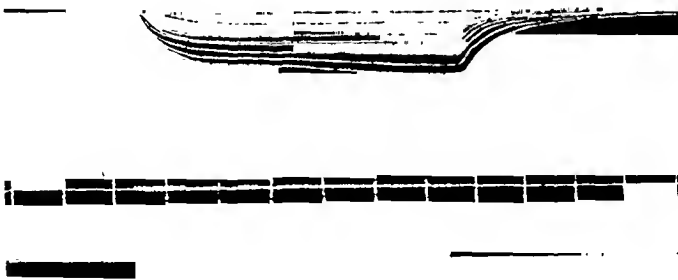


Fig. 3. Isometric contractions of the nictitating membrane on stimulation of the cervical sympathetic with the following frequencies: 0, 0.9, 2.6, 4, 5.1, 8.8, 12 and 17 supramaximal shocks per second. Time recorded in 5 second intervals. Magnification, 9. Tension on muscle, 3 grams. A deviation of 1 cm. in the record is equivalent to a tension of 8.5 grams developed by the muscle.

4. *Contraction of the pregnant uterus of the cat.* The hypogastric nerves, cut or crushed above, were stimulated with supramaximal intensity and varying frequencies. The contractions were recorded by the method described by Rosenblueth (1931).

The results are shown in figures 1-E and 4. The numerical test for a hyperbola is the following:

$$a = 4.4; b = 8.1$$

x	y	$(b - y)(a + x)$
0.9	3.0	27.1
2.6	4.0	28.7
5.1	5.0	29.4
8.8	6.0	27.7
12.0	6.5	26.3
21.0	7.0	27.9
Average		27.8

Maximal deviations +1.6; -1.5.

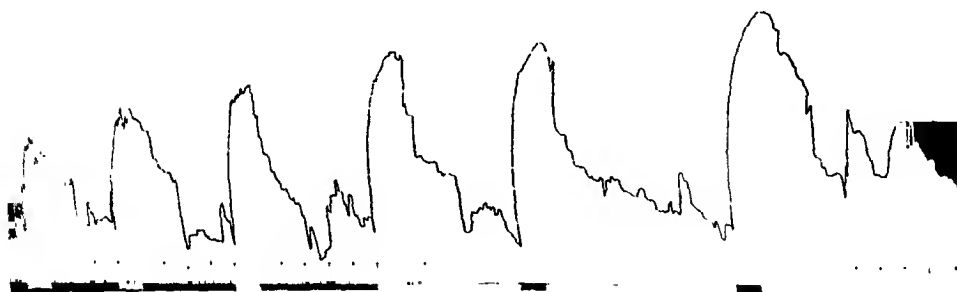


Fig. 4. Isotonic contractions of a cat's pregnant uterus on stimulation of the hypogastric nerves with the following frequencies: 0.9, 2.6, 5.1, 8.8, 12 and 21 supramaximal shocks per second. Time recorded in 1 minute intervals. Magnification, 10. Tension on muscle, 5 grams.

The records are included to show the close similarity of the general shape of the responses with, for example, those of the nictitating membrane, or the stomach on stimulation of the vagus.

5. *Inhibition of the intestine on stimulation of the splanchnics.* The method used to record contraction of the stomach on vagal stimulation (see below) was found inadequate to show inhibition quantitatively. The balloon method was therefore selected. The duodeno-ileum was preferred to the stomach because it presents a higher tonicity during fasting. Anesthetics were avoided because those tried affected the responses. After

dial, for instance, stimulation of the splanchnics or injections of adrenalin evoke a disappearance of peristalsis or rhythmic contraction, but there is no decrease of tone. The cats were therefore pithed (see general method, above). Curare was usually administered; the rhythmic contractions of the intestine decrease slightly in amplitude and frequency, but the tone is not lowered and the responses to nervous stimulation are normal. The adrenals were ligated.

Because of relatively large spontaneous variations of the tone and activity of the organ the quantitation of the responses is not very accurate. A membrane tambour was used as a recording manometer; its calibration with a water manometer revealed a linear direct relation between the deviations of the writing point and the pressures within the range occurring in the experiments. The readings were taken from a mean base line representing tone. Several stimulations were applied with each frequency and averages were obtained.

Figure 1-F represents the curve of a favorable instance. The numerical test is the following.

$$a = 3.1; b = 33.4$$

x	y	$(b - y) (a + x)$
0.8	11	87.4
2.6	18	87.8
4.0	21	88.0
5.1	23	85.3
8.8	26	88.1
12.0	28	81.5
17.0	29	88.4
Average		85.8

Maximal deviations +2.6; -4.3.

6. *Stimulation of the adrenal gland through the splanchnics.* The quantitation of the amounts of adrenin secreted was effected by recording the contractions of the sensitized, denervated nictitating membrane (Rosenblueth and Cannon, 1932) or the rises of blood pressure in the animal sensitized by cocaine. The first test is more exact because in the second important vasoconstrictor influences may increase the response, although in some cases this complication was partly avoided by cutting some of the branches of the corresponding semilunar ganglion. The Elliott (1912) preparation was found unsatisfactory because of the low blood pressure. The following technique makes the changes of blood pressure as sensitive and accurate to adrenin as Elliott's: dial anesthesia is given, the vagi are cut, and cocaine (about 8 mgm. per kilo) is injected intravenously.

The results obtained with either the nictitating membrane or the blood pressure are identical. Figures 5 and 1-G exemplify an experiment with the blood pressure as the indicator. The following is the test of the curve:

$$a = 0.55; b = 5.1$$

x	y	$(b - y) (a + x)$
1.0	0.4	7.3
1.5	0.9	8.4
2.0	1.6	9.0
3.2	2.6	9.4
5.0	3.6	8.3
7.7	4.2	7.4
12.0	4.4	8.8
Average.....		8.4

Maximal deviations +1; -1.1.

The nictitating membrane permits testing the similarity between the curves obtained by stimulations of the splanchnic with varying frequencies

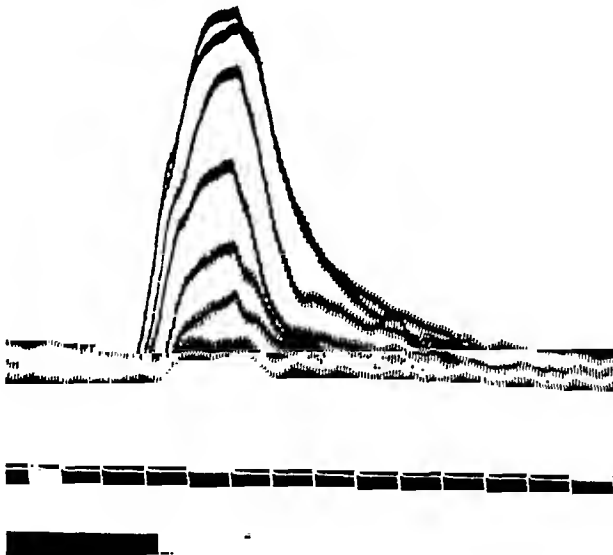


Fig. 5. Cat. Dial, 0.9 cc. per kilo by stomach tube; cocaine, 8 mgm. per kilo intravenously. Vagi cut. Rises of blood pressure on stimulation of the left adrenal medulla through the splanchnics (other branches cut) with the following frequencies: 1, 1.5, 2, 3.2, 5, 7.7 and 12 supramaximal shocks per second. Time recorded in 5 second intervals.

and by injection of varying doses of adrenalin. Figure 1-H illustrates the satisfactory check obtained.

From these data it is possible to calculate the absolute amounts of adrenin secreted by one gland for each stimulus. This amount is 0.0000048 mgm. for this particular experiment and coincides with the average of three observations on different animals.

The maximal amount of adrenin found by Cannon and Rapport (1921) to be secreted reflexly was 0.0037 mgm. per kilo per minute. This figure implies about 10 nerve impulses per fiber per second for a cat of average weight (3.5 kilos). This figure agrees with recent research on the physiological rate of discharge of sympathetic nerves (cf., for instance, Adrian, Bronk and Phillips, 1932).

In the following numerical test of curve 1-H all the points are taken, both those corresponding to stimulation of the splanchnics and those obtained by the injections of adrenin. Now x represents either frequency per second or amounts of adrenin in conventional units ($1 = 0.000286$ mgm.). The asterisks mark the frequencies.

$$a = 1.4; b = 9$$

x	y	$(b - y)(a + x)$
1.75	2.8	19.5
*2.6	2.5	26.0
3.5	5.3	18.1
*4.0	5.0	21.6
*5.1	5.8	20.8
7.0	6.3	22.7
8.7	6.6	24.2
*8.8	7.0	20.4
*12.0	7.6	18.8
17.5	7.7	24.6
*21.0	8.0	22.4
Average.....		21.7

Maximal deviations +4.3; -3.6.

B. STRUCTURES INNERVATED BY THE PARASYMPATHETIC. 1. *Contraction of the stomach.* Cats (fasting 24 hours) were used, usually under dial anesthesia. The splanchnics were cut. The vagi, crushed or cut above, were stimulated immediately below their entrance into the abdomen. The movements were recorded by the method described by Rosenblueth (1931). Notwithstanding the complicated arrangements of gastric muscle fibers, the movements of the writing lever can be satisfactorily interpreted since the main component of the rise is the contraction of the circular

muscle rings at the level where the thread to the lever is attached. The rise of the lever is therefore lineally proportional to the shortening of the radius of this circle, or to the circumference, or, finally, to the actual shortening of the circular muscular fibers.

The results of a typical case are illustrated in figures 6 and 1-I. Again there is a striking similarity between these records and those obtained with the uterus or with the much simpler nictitating membrane.

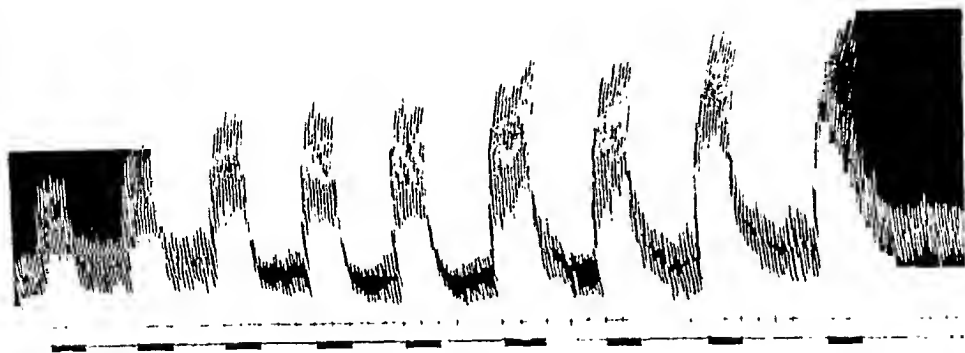


Fig. 6. Isotonic contractions of the circular muscle fibers of the stomach on stimulation of the vagi in the abdomen with the following frequencies: 0.9, 1.5, 2.6, 4, 5.1, 8.8, 12, 17 and 21 supramaximal shocks per second. Time recorded in 30 second intervals. Magnification, approximately 7. Tension on muscle, approximately 6 grams.

The test of curve 1-I for a hyperbola yields:

$$a = 1.7; b = 7.2$$

<i>x</i>	<i>y</i>	(<i>b</i> - <i>y</i>) (<i>a</i> + <i>x</i>)
0.9	2.3	12.7
1.5	2.8	14.0
2.6	4.2	12.9
4.0	5.0	12.5
5.1	5.3	12.9
8.8	5.8	14.7
12.0	6.0	16.4
17.0	6.5	13.1
21.0	6.6	13.6
Average.		13.6

Maximal deviations +2.8; -1.1.

2. *The action of the vagus on the heart.* Both stellates were removed in a cat under dial. The vagi were cut in the neck and shielded electrodes

were placed distally on each nerve. The rate of the heart was taken by means of a carotid cannula connected to a mercury manometer. Supra-maximal stimuli at varying frequencies produced comparable results with either vagus, though quantitatively greater for the right one.

Figure 1-J shows the results obtained. The test of the curve is the following:

$$a = 0.6; b = 31.2$$

x	y	$(b - y) (a + x)$
0.8	7.0	33.9
1.4	12.0	38.4
2.0	16.5	38.2
2.6	20.0	35.8
3.3	22.5	33.9
4.0	24.0	33.1
5.1	25.0	35.3
8.8	27.0	39.5
11.0	28.0	37.1
15.0	29.0	34.3
Average.....		35.9

Maximal deviations $+3.6$; -2.8 .

3. *The submaxillary gland.* Dogs were usually employed because the larger amounts of saliva obtained decreased the experimental error. Cats, however, presented similar results.

Figures 7 and 1-K show the results of a typical observation. The test of the curve is the following:

$$a = 4.37; b = 52.5$$

x	y	$(b - y) (a + x)$
0.8	4.0	250
1.5	7.0	267
2.6	12.0	280
3.3	17.0	273
4.0	21.0	265
5.1	24.5	263
8.8	32.5	264
12.0	36.0	270
16.8	40.0	265
21.0	42.0	267
25.0	43.0	279
Average.....		267

Maximal deviations $+13$; -17 .

The lingual nerve was cut above and below the emergence of the chorda tympani and the latter was stimulated with supramaximal intensities and varying frequencies. Wharton's duct was cannulated and connected with a long tube filled with water (colored by methylene blue) in order to obtain drops of the same size. The drops were recorded by making them fall on a lever attached to a receiving tambour.

The similarity between these results and those obtained in muscle (say, the nictitating membrane) is striking. With the increasing frequency of stimuli the response is earlier, the after-effect longer. The shape of the after-effect in relation to time is shown in figure 1-L; it is an exponential of the formula $y = ke^{-k't}$, identical with the formula of relaxation of smooth muscle (Rosenblueth, 1932).

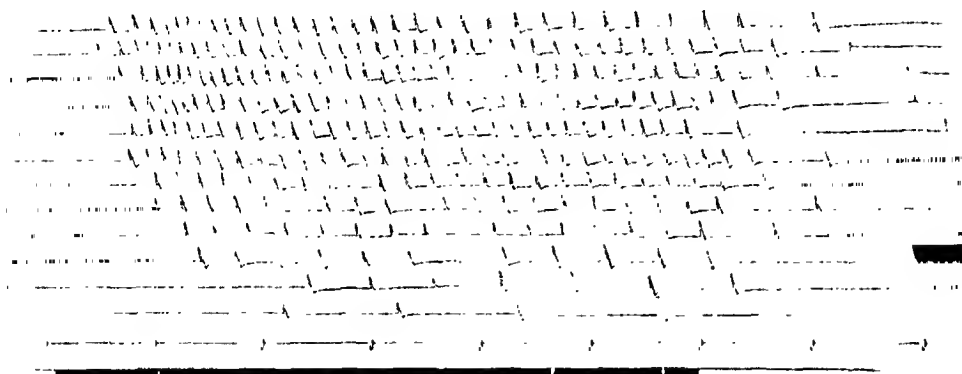


Fig. 7. Dog. Drops of saliva obtained on stimulation of chorda tympani with the following frequencies, from below upward: 0.8, 1.5, 2.6, 3.3, 4, 5.1, 8.8, 12, 16.8, 21 and 25 supramaximal shocks per second. Time recorded in 5 second intervals. At the higher frequencies (from 8.8 on) the last drops do not appear on this record. Twelve drops = 1 cc.

DISCUSSION. In the following discussion it will be shown, first, that the commonly accepted interpretations are not adequate to explain the data presented. The hypothesis of a chemical mediation will then be examined and some of its consequences will be stressed. Some particular instances will be dealt with separately.

The experiments performed on muscles have been dealt with by previous workers under the heading of summation of the responses, a steady state of contraction (tetanus) being interpreted as a fusion of single twitches. It is shown in this paper that the relations between the frequency of the nervous stimuli and the magnitude of the responses obey a general hyperbolic law. This law applies to as widely different types of responses as contraction, inhibition, secretion and the rate of the heart. The only exception found to the law, adrenal secretion, will be discussed later.

If a simple hypothesis, applicable in all cases, will explain all the phenomena observed, it should be preferred to any explanation which can cover only one of the cases. This consideration alone is sufficient to lessen the value of such theories as make a peculiar characteristic of the structure considered (say, the muscle) the basis on which they rest. They will, however, be included in this discussion.

Before discussing summation of responses it is, however, necessary to consider whether the phenomenon of summation of nervous stimuli is not concerned in these cases. The affirmation that the structures innervated by the autonomic nervous system respond only to repeated nervous stimuli occurs frequently in the literature (see, for instance, Lapicque and Meyerson, 1921; Lapicque, 1925; Fredericq, 1928; Bremer and Homès, 1931). We agree with Bishop and Heinbecker (1932) that this statement is incorrect. As shown by Stewart (1900), the cat's bladder contracts sharply in response to a single induction shock. In our experiments the nictitating membrane and the pilomotor reacted definitely to single shocks. So did the heart to vagal inhibition. In the case of the submaxillary gland stimuli separated by as much as three seconds will evoke a definite response if repeated sufficiently. The repetition is necessary to make the secretion apparent because of the minute amounts which would be produced by each stimulus, for the intervals are probably long enough to exclude summation. In fact, a response was invariably obtained in every structure examined with frequencies as low as one shock every one or two seconds. This makes it improbable that summation of stimuli played a rôle in any case. Finally, even if it did, whatever the theory adopted for the summation of nervous stimuli, the only consequence in relation to the responses to varying frequencies would be a change of scale. This improbable possibility will therefore be disregarded.

Fulton (1925) explained summation in muscle as due to the viscosity of the tissue and its changes during contraction (see Gasser and Hill, 1924). This theory is inadequate for either skeletal or smooth muscle because, if it were correct, the maximal tension possible would always be attained, independently of the frequency, after a lower frequency limit, necessary for summation to occur at all, has been reached. The only difference between slower and faster rates of stimulation would be that the former would produce a maximal tension after a longer period of application than the latter. This consequence of the theory is in disagreement with all experimental evidence.

Mines (1913) proposed the hypothesis that summation in skeletal muscle is due to an increased CH (quantal releases of lactic acid at each stimulus). The contraction would then be proportional to the CH of the muscle. This is a chemical mediation theory. It is unacceptable because of the large amounts of lactic acid which would be necessary to change the pH. Be-

sides, it leads to a linear or parabolic relation between the frequencies and the responses (cf. any work on physical chemistry), which is not confirmed by experience.

Hill (1913) showed that, although heat-production and tension increase with frequency, the heat-production per unit of tension developed is constant. He therefore concluded that the rise of tension is due to the presence of chemical substances, liberated in conjunction with heat by the processes called forth by excitation. The amount of tension developed is proportional to the amount of these substances present. This theory was again taken up by Hartree and Hill (1921) and slightly enlarged and modified, as follows. Let contraction be due to the appearance of substance B (lactic acid) at certain surfaces of the muscle. In relaxation B is destroyed or removed from its seat of action. B is produced from A, of which there is a large supply in the muscle, according to the reaction $A \rightarrow B$, under the influence of a catalyst. Equilibrium exists in a space impermeable to B. A shock produces a certain momentary permeability. If now the shock is repeated the amount of B which passes out is smaller. Equilibrium will occur independently of the frequency when all B has escaped, and is conditioned only by the speed of the reaction $A \rightarrow B$.

The hypothesis is not very explicit as regards our present problem. If the last statement be taken textually the assumption is inadmissible, for equilibrium (i.e., sustained constant tension during stimulation) is dependent on frequency of stimulation (see, for references on skeletal muscle, Davis and Davis, 1932). The amounts of B diffusing at each shock will only be smaller with successive stimuli if the frequency is greater than the velocity of the reaction $A \rightarrow B$. If the only factor were this velocity, regardless of frequency, equilibrium would always be attained at the same amount of tension (i.e., the maximal tension would always be reached) and this is not the case.

The hypotheses examined do not, therefore, agree with experimental evidence. Before analyzing the theory of a chemical mediation it now becomes necessary to examine the part the nervous impulses elicited by the electrical stimuli may play in explaining the curves obtained. This can be only minimal and negligible. Gerard, Hill and Zottermann (1927) found a decrease of the electrical response and the heat produced for a single nervous impulse (and therefore also probably the "intensity" of the impulse) as the frequency increased. At the rates used in these experiments however (i.e., successive impulses occurring well beyond the refractory period of the nerve), this decrease can be neglected. The effective inflexion toward the asymptote in Gerard, Hill and Zottermann's curve occurs when the limits of the refractory period are reached.

The theory of a chemical mediation adopted here can be expressed as follows. Each quantal (all-or-none) nervous impulse liberates a quantal

(constant) amount of a chemical mediator, M. M combines with some hypothetical substance H in the effector according to the reaction $M + H \rightleftharpoons MH$. The response is proportional (not all-or-none) to the amount of MH formed. Free M is destroyed locally; this will produce the direction \leftarrow of the reaction when no more M is supplied and relaxation will ensue. The amount of M which can be destroyed locally at a given time is limited; hence if M should occur at a higher concentration than this amount it will diffuse into the blood where it may be destroyed or whence it may diffuse into other structures and produce its characteristic effects.

The hypothesis leads to the following formula for the relations between the height of the response R and the concentration of (M):
$$R = \frac{(M)}{k - k'(M)}.$$

But $(M) = qF$ (frequency). Hence,
$$R = \frac{F}{k - k'F}.$$
 This is the formula

for a rectangular hyperbola whose asymptotes are parallel to the axes. It has been shown in the data presented in the foregoing pages how fully experimental evidence confirms these relations.

The shape of the responses, the increasing after-effects with higher frequencies, the constant linear rise of the responses with frequencies higher than a certain critical level, are all accounted for satisfactorily. The direct evidence in favor of the adequacy of the explanation is the demonstration of autonomic effects in denervated organs after autonomic stimulation elsewhere: vagal and sympathetic substances (Loewi, 1921), sympathin (Cannon and Bacq, 1931), chorda-hormone (Babkin, Stavratsky and Alley, 1932). The after-effects are longer in stimulation of the sympathetic (see figs. 2 and 3) or of the chorda tympani (see fig. 7) than in stimulation of the vagal territory (heart, stomach, see fig. 6 and pp. 26-28). This would indicate a more ready destruction of the mediator in the latter system. It is interesting to correlate this fact with Freeman, Phillips and Cannon's (1931) fruitless attempt to show a vagus hormone in conditions similar to those in which sympathin is readily demonstrated.

The effect (see p. 16) of excessive frequencies of stimulation (i.e., a maximal response followed shortly by a slow and continuous fall) coincides with that found by Orías (1932) on the nictitating membrane and by Davis and Davis (1932) on skeletal muscle. Its interpretation need not be discussed here. It is evident that after a certain frequency the refractory period of the muscle, or of the ganglion if preganglionic fibers are stimulated, probably increased by previous stimulation, will play a definite part in producing the effect.

Bishop and Heinbecker (1932) state that they do not believe that accurately interpretable results can be obtained by stimuli on the cervical sympathetic more frequent than 10 per second. Our experiments (see

figs. 1-B and C, 2 and 3) would increase this limiting frequency to 20 per second for the preganglionic fibers in the cervical sympathetic. These figures agree with the values found by Bishop and Heinbecker (1930) for the refractory periods of the ganglion synapse and the postganglionic fibers (20 to 30 and 4.5σ , respectively). We agree with these authors (1932) that the results of Querido (1924) and Veach and Pereira (1925) with much higher frequencies are unacceptable.

The approximate frequencies found to produce a maximal response in our experiments were, for the preganglionic fibers, the following:

STRUCTURE	FREQUENCY PER SECOND
Sympathetic	
Pilomotors.....	15
Nictitating membrane.....	20
Pregnant uterus (postganglionic).....	20
Intestine.....	20
Adrenal medulla.....	25
Heart (probably postganglionic).....	25
Parasympathetic	
Heart.....	30
Submaxillary gland.....	36
Stomach.....	25

The maximal frequency effective on the preganglionic fibers is the only one that is interesting since, as Bishop and Heinbecker (1932) show, the longer refractory period of the ganglion will be the determinant of this maximal frequency, and there is no after-discharge from the ganglion.

The chemical theory adopted makes the process of relaxation an active one: it is rather a progressive "decontraction" conditioned by the gradual disappearance of the mediator than an actual sudden relaxation, the shape of which would then be conditioned by the viscosity of the muscle. This view is corroborated by the increasing after-effects with higher frequencies (see figs. 2, 3, 4 and 6).

This concept of the disappearance of the response is undoubtedly true in the case of the submaxillary gland (see fig. 7). The rate of the disappearance here as elsewhere is an exponential (see fig. 1-L). The interpretation of the responses of the submaxillary gland must, however, be dealt with more extensively. The hyperbola presented in figure 1-K was obtained by plotting the total amounts of saliva produced against the frequency. This total amount is not comparable with the maximal height

of the contraction of a muscle, but rather with the area of the record of a contraction. These areas can be readily obtained with a planimeter. Their relations to the frequency or to the amounts of chemical mediator or of adrenin injected can be obtained by integrating the formula for contraction (see Rosenblueth, 1932)

$$y = \mu \left[\frac{k(M)X}{k' + k(M)} (1 - e^{-(k' + k(M))t}) \right]$$

between the limits 0 and t_1 (end of contraction), and the formula for relaxation

$$y = (MH)_0 e^{-k't} = \mu \frac{k(M)X}{k' + k(M)} e^{-k't}$$

between the limits t_1 and ∞ , and adding. Calling A the area we then have

$$\begin{aligned} A &= \int_0^{t_1} \mu \left[\frac{k(M)X}{k' + k(M)} (1 - e^{-(k' + k(M))t}) \right] dt + \int_{t_1}^{\infty} \mu \frac{k(M)X}{k' + k(M)} e^{-k't} dt = \\ &= \frac{\mu k(M)X}{k' + k(M)} \left[t_1 + \frac{e^{-k't_1}}{k'} + \frac{1}{k' + k(M)} (1 + e^{-(k' + k(M))t_1}) \right] \end{aligned}$$

If we now consider that t_1 varies only slightly and in the same direction as M , and that M is exceedingly small compared to the other values concerned (see, for instance, the figures obtained for the output of adrenin by the adrenal gland per stimulus (p. 26); M would probably be still smaller), we can consider the expression in the parenthesis to be practically constant. The formula then becomes

$$A = \frac{(M)}{k' - k(M)}$$

that is, it again yields a hyperbola.

This is what curve 1-K illustrates for the salivary gland. This procedure was likewise applied to several curves obtained from contractions of the nictitating membrane to varying frequencies of stimulation or to varying doses of adrenin. The areas of these contractions, which represent the work done, produced similar curves.

If, on the other hand, tension in muscle be legitimately compared to flow in the salivary gland, it is sufficient to plot the number of drops for a given time during stimulation, or the intervals between these drops, against the frequencies, to make the situation comparable to that of the muscle. If this is done hyperbolas are again obtained. The total response

was used, and not these latter methods, because of simplicity and greater accuracy in quantitating the response, since the flow is not constant. The fact that the first drops occur at a faster rate than the subsequent ones can possibly be explained as due to squeezing of saliva from the ducts by vasodilatation. The flow later (after about 30 seconds) becomes steady. It requires very long stimulation (over 10 minutes) for the exponential decrease due to fatigue to appear.

The all-or-none law is rejected for smooth muscle by the interpretation adopted. The same conclusion was reached in the case of the action of adrenin (Rosenblueth, 1932). In this relation we would mention that Bishop and Heinbecker (1932) repeatedly state that smooth muscle does not follow the all-or-none principle, without, however, giving any evidence, arguments, or references.

The secretion of the adrenal medulla offers an interesting exception to the hyperbolic law. It was shown (Rosenblueth, 1932) that varying concentrations of adrenin in the blood bear a hyperbolic relation to the responses of the indicator. Since varying frequencies of stimulation of the splanchnics again elicit reactions of the indicator which yield a hyperbola (see figs. 1-G and H, and 5 and pp. 25, 26) it follows that the amounts of adrenin secreted are linearly proportional to the frequency. This atypical behavior is possibly correlated with the atypical innervation of the gland, by preganglionic fibers only (Elliott, 1913), and with the embryological identity of the adrenal medulla and the postganglionic neurones. In accord with this suggestion the production of the mediator might depend on the postganglionic neurone.

Bishop and Heinbecker (1932) state that no difference can be detected by observation of the end response when, beginning with a submaximal intensity, either the frequency or the intensity of stimulation of the cervical sympathetic is increased. From this they conclude that temporal and spatial summation amount to the same values. We must disagree with this conclusion. If the optimal frequency is chosen and the intensity increased the maximal response will be obtained, but it will be due to spatial *plus* temporal summation. If, however, the frequency is low, say 2 per second in any of our experiments, spatial summation alone will never produce the maximal response.

Spatial and temporal summation can only be legitimately compared when acting independently, but this comparison cannot be done experimentally. Exclusive temporal summation is simple to analyze; the pilomotor responses are a good example of it since the muscles are probably innervated by a single fiber (see p. 16). But the effects of pure spatial summation will vary with the number of fibers innervating the structure considered. It can, however, be stated that the maximal effects of pure spatial summation in the autonomic nervous system can be considerably increased if temporal

summation comes also into play. The maximal height of contraction of the nictitating membrane obtained by stimulation at the optimal frequency is usually 10 to 15 times greater than that of a "twitch" in response to a single supramaximal stimulus. In the case of the submaxillary gland this ratio is obtained by comparing the flow of saliva during stimulation at the optimal frequency and after a single supramaximal shock (see p. 34). In one experiment the flow was 0.066 drop per second when stimulated at the frequency of 1 per second and 1.25 drop per second when the frequency of stimulation rose to 30. This would give a ratio of 19, which could be still increased by slowing further the lower frequency.

Our conclusion is then that spatial summation in the autonomic nervous system is of scant value if compared with the effects of temporal summation.

SUMMARY AND CONCLUSIONS

The relations between the responses and the frequency of supramaximal stimulation of autonomic nerves were studied in the following cases: a single hair in the tail of the cat (see fig. 1-A and pp. 14-18), the nictitating membrane (isometric and isotonic contractions, see figs. 1-B and C, 2 and 3 and pp. 18-21), sympathetic acceleration of the heart (see fig. 1-D and p. 21), isotonic contractions of the cat's pregnant uterus (see figs. 1-E and 4 and p. 28), inhibition of the duodenum (see fig. 1-F and p. 24), the adrenal medulla (see figs. 1-G and H and pp. 24-26); vagal stimulation of the stomach (see figs. 1-I and 6 and pp. 26, 27), vagal inhibition of the heart (see fig. 1-J and p. 28), and the submaxillary gland (see figs. 1-K and 7).

The shapes of the records of the muscular responses are exponentials, with the formulae $y = k(1 - e^{-k't})$ for the contraction and $y = ke^{-k't}$ for the relaxation (see figs. 2, 3, 4 and 6). The second formula is also applicable to the rate of disappearance of the response of the submaxillary gland after stimulation (see fig. 1-L).

With the exception of medulliadrenal secretion, which is in a linear relation with the frequency (see p. 35), all the systems investigated obey the following hyperbolic law $R = \frac{F}{k' - kF}$, where R is the maximal height of the response, F the frequency of stimulation, and k' and k , constants (see figs. 1-A to K).

Summation of nervous stimuli probably does not occur in these experiments (see p. 30).

Several theories of summation of responses, proposed by various authors, are examined and found inadequate to explain the experimental data presented in this paper (see pp. 30, 31).

The characteristics of the nervous impulses elicited by the stimuli probably do not account for the phenomena observed (see p. 31).

The following chemical hypothesis is proposed. Each quantal nervous impulse liberates a quantal amount of a chemical mediator, M . M combines with some substance H in the effector according to the reaction $M + H \rightleftharpoons MH$. The response is proportional to the concentration of MH , not all-or-none. Free M is destroyed locally, hence relaxation. This destruction occurs at a limited rate, hence possible diffusion to other structures when the concentration exceeds this limit.

This hypothesis accounts for all the data observed: the exponential shape of the responses, the hyperbolic ratio between the maximal heights and the frequencies, the constant linear rise of the responses with frequencies higher than a certain critical level (see figs. 2 and 3 and p. 21), and the increasing after-effects with increasing frequencies (see figs. 2, 3, 4 and 6 and p. 21). The autonomic effects in denervated organs after autonomic stimulation elsewhere in the organism are direct evidence which supports the hypothesis (see p. 32 for references).

The average optimal frequencies found in these experiments are given on page 33.

It is shown that relaxation of smooth muscle is probably an active process, a "decontraction," comparable to the disappearance of the salivary flow after stimulation of the chorda tympani (see fig. 1-L and p. 33).

The area of a contraction is obtained by integration of the corresponding formulae. This area represents the work performed, and is comparable to the total amount of saliva secreted during stimulation. It is shown that there exists again a hyperbolic relation between the contraction areas or the total amounts of saliva and the stimulation frequencies (see fig. 1-K and pp. 33, 34).

Spatial and temporal summation in the responses of organs supplied by the autonomic nervous system is discussed in pages 35, 36. It is concluded that the effects of spatial summation are small when compared with those obtained by temporal summation.

BIBLIOGRAPHY

- ADRIAN, E. D., D. W. BRONK AND G. PHILLIPS. 1932. *Journ. Physiol.*, lxxiv, 115.
BABKIN, B. P., G. W. STAVRAKY AND A. ALLEY. 1932. *This Journal*, ci, 2.
BISHOP, G. H. AND P. HEINBECKER. 1930. *Ibid.*, xciv, 170.
1932. *Ibid.*, c, 519.
BREMER, F. AND G. HOMÈS. 1931. *Mem. de l'Acad. Roy. de Belgique*, xi, 8.
CANNON, W. B. 1931. *Endocrinol.*, xv, 473.
CANNON, W. B. AND Z. M. BACQ. 1931. *This Journal*, xcvi, 392.
CANNON, W. B. AND S. W. BRITTON. 1925. *Ibid.*, lxxii, 283.
CANNON, W. B. AND D. RAPPORT. 1921. *Ibid.*, lviii, 308.
DAVIS, H. AND P. A. DAVIS. 1932. *Ibid.*, ci, 339.
ELLIOTT, T. R. 1904. *Journ. Physiol.*, xxxi, 21.
1912. *Ibid.*, xlv, 374.
1913. *Ibid.*, xlvi, 285.

- FREDERICQ, H. 1928. *Physiol. Rev.*, viii, 501.
- FREEMAN, N. E., R. A. PHILLIPS AND W. B. CANNON. 1931. *This Journal*, xcvi, 435.
- FULTON, J. F. 1925. *Ibid.*, lxxv, 211.
- GASSER, H. S. AND A. V. HILL. 1924. *Proc. Roy. Soc., B*, xcvi, 398.
- GERARD, R. W., A. V. HILL AND Y. ZOTTERMAN. 1927. *Journ. Physiol.*, lxiii, 130.
- HARTREE, W. AND A. V. HILL. 1921. *Ibid.*, lv, 133.
- HILL, A. V. 1913. *Ibid.*, xlvii, 305.
- LAPICQUE, L. 1925. *Ann. Physiol. et Physicoch. Biol.*, i, 132.
- LAPICQUE, L. AND I. MEYERSON. 1921. *C. R. Soc. Biol.*, lxxii, 63.
- LOEWI, O. 1921. *Pflüger's Arch.*, clxxxix, 239; exciii, 201.
- MINES, G. R. 1913. *Journ. Physiol.*, xlvi, 1.
- ORFAS, O. 1932. *This Journal*, cii, 87.
- QUERIDO, A. 1924. *Ibid.*, lxx, 29.
- ROSENBLUETH, A. 1931. *Ibid.*, xcvi, 186.
1932. *Ibid.*, ci, 149.
- ROSENBLUETH, A. AND W. B. CANNON. 1932. *Ibid.*, xcix, 398.
- STEWART, C. C. 1900. *Ibid.*, iv, 185.
- VEACH, H. O. AND J. R. PEREIRA. 1925. *Journ. Physiol.*, lx, 329.

THE EFFECT OF PROGESTIN-CONTAINING EXTRACTS OF CORPORA LUTEA ON UTERINE MOTILITY IN THE UNANESTHETIZED RABBIT WITH OBSERVATIONS ON PSEUDO-PREGNANCY¹

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In a previous series of experiments one of us has shown, by the uterine fistula in the unanesthetized rabbit, that the uterus usually undergoes strong, rhythmical contractions when the animal is in heat³ and that within a few hours after copulation this motility gradually decreases in intensity so that by the time ovulation has occurred, or even before, the uterus is quiescent (Reynolds and Friedman, 1930a, 1930b). This period of inactivity persists throughout pseudopregnancy, but as the doe again comes into heat, rhythmical motility returns. After castration the motility also disappears within 2 to 3 days, but if Theelin is injected intravenously into the recently castrated animal, as little as 2 to 5 r.u. per kilo causes a return of motility within 24 hours (Reynolds, 1931a). This series of experiments shows that rhythmical motility is present when does are in heat, and that in castrated does the injection of Theelin restores this motility. The natural conclusion is that the motility is due to the oestrin present in the animal and that it is one of the physiological manifestations of oestrus. It was later found that, whereas the quiescent uterus of the castrate may be

¹ The previous papers of the series on the hormone, progestin, are listed in the bibliography as follows: I, Corner, 1928; II, Corner and W. M. Allen, 1929; III, Allen, W. M. and Corner, 1929; IV, Goldstein and Tatelbaum, 1929; V, W. M. Allen, 1930a; VI, W. M. Allen, 1930b; VII, Allen, W. M. and Corner, 1930; VIII, Allen, W. M., 1932. Previous work on uterine motility in the unanesthetized rabbit has been summarized in a short article by one of us (S.R.M.R.) which appeared in *Endocrinology*, xvi, 193, 1932.

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I am indebted to Doctor George L. Streeter, Director of the Department of Embryology, Carnegie Institution of Washington, for extending to me the privilege and full use of the excellent facilities of that laboratory throughout this year.

³ The term "heat" or "oestrus" as used in this paper refers to a condition in the female rabbit when a sexually mature doe is, in addition to being willing to mate, able to ovulate in response to the normal stimulus of coitus (see Reynolds, 1931a, p. 715).

restored to oestrous motility by the injection of 2 to 5 r.u. of Theelin per kilo, the quiescent uterus of pseudopregnancy is completely refractory to Theelin, even to doses of 1090 r.u. per kilo. This of course indicates that during the pseudopregnancy some substance or condition is present which brings about this tremendous change in susceptibility to Theelin, and that perhaps the inhibiting substance or mechanism originates in the corpus luteum. This is borne out by the fact that removal of the ovaries (hence, corpora lutea) at this time renders the uterus susceptible to small amounts (5 r.u. per kilo) of Theelin (Reynolds, 1931b). Regardless of the simultaneous occurrence of this inhibition of motility and the presence of corpora lutea, however, there have been no experiments which directly show that the inhibitory substance is produced by the corpus luteum.

In another series of papers the other of us, in collaboration with Dr. G. W. Corner, has described the preparation of active corpus luteum extracts which, when injected into rabbits castrated 18 to 24 hours after mating, regularly produce progestational proliferation of the endometrium and bring about normal implantation of the blastocysts and development of the fetuses even to full term. These extracts contain, therefore, a hormone which produces in the castrated rabbit cytological and physiological conditions identical with those found during pregnancy or pseudopregnancy, and because of this fact, the hormone was named progestin, i.e., a substance which favors gestation.

The problem in this paper is to ascertain whether or not accurately assayed progestin-containing extracts will inhibit normal oestrous motility following injections of Theelin and, if such inhibition is observed, to obtain some evidence if possible on the nature of the substance responsible for the inhibition.

The corpus luteum extracts used in this work were made by the method previously described. The corpora lutea of swine are extracted with boiling neutral alcohol. The alcohol is distilled off and the residue extracted with ether. The phospholipids are removed from the ether solution by precipitation with acetone and the acetone-ether soluble oil thus obtained is freed from cholesterol and a large amount of neutral fat by freezing from 70 per cent methyl alcohol. The methyl alcohol soluble substances, after removal of the methyl alcohol, are extracted with ethyl ether and the ether washed with sodium bicarbonate solution. One rabbit unit of progestin prepared by this method usually is equal to about 6 to 8 mgm. of solids. The specific corpus luteum extracts used were of two distinct degrees of purity. Extracts 87 and 92 were so-called crude oil, fraction i; 1 rabbit unit = 400 mgm.) and extract 90 was more highly purified, fraction o; 1 rabbit unit = 8 mgm.) (W. M. Allen, 1932b). Extracts 87 and 92 produced mucification of the vaginas of castrate mice when a suitable dose was injected for 8 days, and cornifica-

tion when larger doses were given. Extract 90 was not tested for its oestrin content, but other extracts made in exactly the same manner have produced either mucification or cornification depending on the dose. These extracts, therefore, contained at least two well-known hormones, progesterin and oestrin (Meyer and Allen, 1932).

The Theelin (50 r.u. per cc.) used in these experiments was supplied by Parke, Davis & Co., and has been shown to be a chemically pure substance having the empirical formula $C_{18}H_{22}O_2$. This crystalline hormone was originally isolated by Veler, Thayer and Doisy (1930) and independently by Butenandt (1929) and Laqueur (1930). Another, more definitive name, ketohydroxyoestrin, has recently been proposed for this hormone by Marrian (1932).

EFFECT OF PROGESTIN ON SPONTANEOUS OESTROUS MOTILITY (table 1, fig. 1). For the study of progesterin-containing extracts on normal motility 10 oestrous (post partum) rabbits were used whose ovaries were intact and whose uterine motility records were of the normal oestrous type at the time injections were begun. They were then injected subcutaneously once daily for 2 to 3 days and records of the uterine motility made 24 hours after each injection, and daily after injections were stopped. From perusal of table 1 it is seen that the marked pre-injection motility was much diminished 24 hours after the first injection in most cases, although some animals showed arrhythmical motility, and that upon a second and a third injection the motility was still further reduced and in some cases the uterus was quiescent. Normal motility reappeared from two to five days after stopping the injections of progesterin. One aspect of the effect of progesterin on spontaneous motility is not revealed in table 1. Records of motility were made in most of the above instances at the intervals of three, eight and twelve hours after the subcutaneous injection of progesterin. It was found in all cases but one that nearly complete quiescence was attained in the interval of eight to twelve hours after the injection. A slight increase occurred by twenty-four hours (the time first noted in the table) after the injection, and so table 1 indicates motility which is really a partial recovery from the first injection. This motility was in turn overcome by the subsequent injections of progesterin. This was especially exemplified by rabbits 22 and 28, which from the table appear to have barely responded to the first injection, yet the uteri of these animals showed only feeble motility some five to ten hours after the injection of progesterin. Owing to this wide variation in the time of appearance of diminished motility and in the degree of response we do not attach much importance to the absolute time of first appearance of the inhibitory effect of progesterin, for admittedly the mode of administration is artificial and there is as yet no reason to suppose that the single injection does more than roughly approximate the normal rate of formation of progesterin by the corpus luteum. The significance of the

TABLE 1
Effect of progestin on spontaneous motility of the uterus

Types of activity may be designated in the following manner:

- 0 = Quiescent condition of uterus in which no activity can be recorded with our system.
 + = Feeble activity is that which with our system of recording gives records of contractions of $\frac{1}{2}$ inch or slightly less.
 ++ = Moderate activity is that which varies between $\frac{1}{2}$ inch to 2 inches, but which appears to average 1 inch to $1\frac{1}{2}$ inches.
 +++ = Marked activity is that which varies between 2 inches to 4 inches.
 ++++ = Marked activity, over 4 inches.

In all these classes of activity one may find rhythmical (regular) activity, or arrhythmical (irregular) activity. Frequently with feeble activity one sees a sort of undulating movement, constant in rate in any one animal for a short period of time, but varying in rapidity from animal to animal.

RABBIT NUMBER	AMOUNT OF PROGESTIN DAILY IN RABBIT UNITS	NUMBER OF DAYS IN- JECTED	MOTILITY BEFORE INJECTION	DAILY MOTILITY 24 HOURS AFTER EACH INJECTION OF PROGESTIN			DAILY MOTILITY 48 HOURS AND LATER FOLLOWING LAST INJECTION OF PROGESTIN			
				1	2	3	1	2	3	4
22	0.2	2	++++ 60"-80"	+++ irregular	+	0-+ irregular	0			
23	0.2	3	++++ 40"-45"	0-+ irregular	++ irregular	+	0-+ irregular	+++ 60"-70"	+++ 50"-60"	
24	0.2	3	++++ 35"-40"	+++ irregular	+++ irregular	irregular chewed fistula (no more rec- ords)				
25	0.2	2	++++ 50"	+++ 35" and ir- regular	+	+			peritonitis; dis- eased records of return of motility	

26	0.2	2	++ irregular	0-+ irregular	0-+ irregular	++ 60"	++ 60"	++ 60"
27	0.2	2	++ 50"-55"	0	+	++ irregular	++ 60"	++ 60"
28	0.2	2	++ 60"	++ irregular	++ irregular	++ 30"-40"	++ irregular	++ 35"-45"
36	1.0	3	++ irregular	++ irregular	0	++ 60"-80"	++ 60"-80"	++ 60"
37	1.0	3	++ 60"	++ irregular	+	++ irregular	++ irregular	++ 60"
38	1.0	3	++ 60"	0-+ irregular	0-+ irregular	++ irregular	++ irregular	++ 35"-45"

Nos. 22-28 treated with purified extract 90 (1 rabbit unit = 8 mgm).
 Nos. 36-38 treated with crude extract 92-A (1 rabbit unit = 400 mgm.).

occurrence of the inhibitory effect before the time when it is known that proliferation occurs will be discussed later.

The uteri of these animals were not submitted to histological study at the termination of the experiment since they were injected with progestin for only 2 to 3 days and since the uterine motility was studied for several days after discontinuing the progestin. Histological study at that time certainly would not have revealed any proliferation because the animals were not injected long enough to induce good proliferation and any proliferation which may have been induced would have disappeared by the time the motility records were completed. A gross examination of the tissues was made in each case however, and it was found that the uteri were free of adhesions, infections and apparent inflammation. One rabbit (no. 25) noted below developed peritonitis following the perforation of the

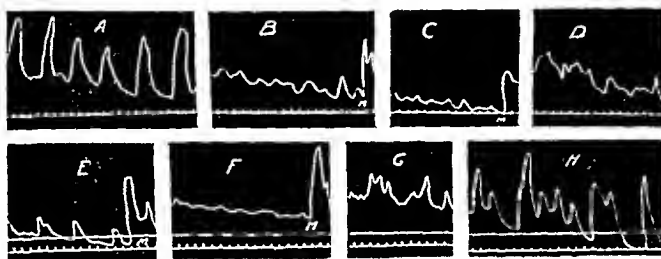


Fig. 1. Records showing the effect of progestin on spontaneous uterine motility in the unanesthetized rabbit. A, normal motility; B, C, D, motility 3, 8 and 12 hours respectively after single subcutaneous injection of progestin (0.2 rabbit unit); E, motility 24 hours after injection, followed by a second and last injection of progestin (0.2 rabbit unit); F, G, H, motility 24, 48 and 72 hours following last injection of progestin. (Rabbit 27, table 1.) $\frac{1}{2}$ size. M, mechanical response to demonstrate patency of balloon recording system.

uterus in the course of inserting the balloon for recording. Data from this doe have been considered as reliable until the time when it was reasonably certain that accidental perforation occurred.

Summary. Subcutaneous injection of progestin-containing extracts into non-castrated rabbits whose uteri exhibit normal oestrous motility causes marked reduction in the motility within 24 hours and if the extract is injected for two or three days complete quiescence may be obtained. Normal oestrous motility returns from two to five days after discontinuing the progestin injections.

EFFECT OF THEELIN IN CASTRATED ANIMALS INJECTED WITH PROGESTIN (table 2, fig. 2). In this group of experiments adult female rabbits were ovariectomized 18 to 24 hours after mating with normal bucks. They were, therefore, identical in all respects with those which have been used for the

routine testing of corpus luteum extracts. They were then injected subcutaneously once daily for five days with oily extracts of the corpus luteum which contained standardized amounts of progestin. The first injection of progestin was made on the day of ovariectomy. They were injected with from 1.6 to 5.0 rabbit units total during the period of 5 days. On the fifth day of injections they were injected intravenously in divided doses over a period of 8 hours with the amount of Theelin desired (100–1000 rat units). The uterine motility was recorded immediately before the injection of Theelin, 10 to 12 hours and 24 to 30 hours after the injection of Theelin. It has been amply shown that if motility is to follow injection of Theelin, it will attain maximum amplitude within 24 hours or less of the time of the first injection. After the last recording of motility the animals were autopsied, the ovarian stumps examined to exclude the presence of remnants of the ovary, and the uteri fixed in Bouin's fluid so that histological study could be made subsequently.

In the first six cases of this series the small amount of progestin (1.6 rabbit units in 5 days) sufficed to prevent motility following the intravenous injection of 100–200 rat units of Theelin per kilo of body weight (nos. 1, 2, 3, 4) but this dose was insufficient to prevent normal oestrous motility following the injection of 500 rat units (nos. 5, 6). This is in direct contrast to the situation in the non-progestin-injected castrated rabbit, for in a five day castrate, 2 to 5 rat units of Theelin per kilo invariably bring about marked uterine motility within 24 hours (Reynolds, 1931a). The injection of corpus luteum extracts containing 1.6 rabbit units of progestin therefore increased the refractoriness of the uterus to oestrin from 40 to 100 times. In the remaining animals of this series larger doses of progestin (4.8 to 5.0 rabbit units) and of Theelin were given. The results differ from the first six animals only in that as much as 1000 r.u. per kilo (4300 rat units total in one case) failed to elicit any motility whatsoever. Rabbit 12, which received a total of 3100 rat units (62 cc.) of Theelin intravenously, died from cardiac failure following the final intravenous injection, but previous to the time of this last injection no sign of a beginning motility response was observed, yet it may frequently be seen by this time in normal animals treated only with Theelin. It would seem, therefore, that this doe resembles the others of this series. Larger doses of Theelin perhaps might overcome this inhibition but it is technically difficult to give more than this amount. However, the doses given are quite comparable to those which have been found to be inhibited in normal pseudopregnancy (Reynolds, 1931b).

The last point to which attention should be called is the complete inability of corpus luteum extracts in which the progestin has been inactivated by treatment with 2 per cent alcoholic KOH to inhibit Theelin motility. Alkali of this strength, even at room temperature, completely

TABLE 2
Motility response of the uterus of the castrated rabbit treated with progesterin, to the intravenous injection of theelin. Controls treated with inactivated progesterin

RABBIT	WEIGHT <i>kgm.</i>	DAYS POST PARTUM <i>days</i>	CASTRATED POST COITUM <i>hours</i>	AMOUNT OF PROGESTERIN AD- MINISTERED IN DAY 5 POST COITUM <i>rabbit units</i>	AMOUNT OF THEELIN ON DAY 5 POST COITUM <i>rat units per kilo- gram body weight</i>	MOTILITY			ENDOMETRIAL PROLIFERATION
						Immediately before theelin	10-12 hours after theelin	24-30 hours after theelin	
1	2.9	no recent litter	24	1.6	100	0	0	+	++
2	2.6	recently pseudo- pregnant	24	1.6	100	0	0	irregular +	++
3	1.8	2	20	1.6	200	0	0	irregular ++	+
4	2.0	1	20	1.6	200	0	0	irregular +	+
5	2.1	2	22	1.6	500	0	+	irregular ++	++
6	2.1	2	22	1.6	500	0	40" ++ irregular	40" ++ 40"	++ ++
7	2.1	4	20	4.8	300	0	0	0	++
8	1.8	2	20	4.8	300	0	0	0	++
9	2.75	6	24	4.8	500	0	0	0-+ very slight	++
10	4.0	recently	20	5.0	500	0-+	0	0	++
11	2.8	no recent litter	20	5.0	1,000	0-+	0	0	++
12	3.1	recently	22	5.0	1,000	irregular	0	see text	++ or ++
13	4.3	6	24	5.0	1,000	+	0	0-+ irregular	++ ++

Controls—progestin inactivated in alkalis									
33	2.5	recently pseudo-pregnant	24	2.5	5	0-+ irregular	++ 50"	++-++ 50"	0
34	3.0	recently pseudo-pregnant	24	2.5	5	0-+ irregular	++ 50"	++-++ 25"	0
35	3.0	recently pseudo-pregnant	24	4.0	5	+ irregular	++ 45"-50"	++-++ 60"-70"	0

Nos. 1 to 9, treated with crude extract 87 (1 rabbit unit. = 400 mgm.).
Nos. 10-13, treated with purified extract 90.
Nos. 33-35, treated with inactivated crude extract, 92-B(92-A treated with KOH).

inactivates progestin (W. M. Allen, 1930b). Five rat units of Theelin per kilo were sufficient to bring about good motility in animals injected with extracts which previous to inactivation had contained 2.5 to 4.0 rabbit units of progestin (see fig. 2). These animals indicate quite conclusively that the inhibiting factor was destroyed with the proliferating factor (progestin). The question of the possible identity of the inhibitory substance with progestin will be discussed later in the paper.

The histological study of the uteri of these animals showed that in general they were remarkably free of infection. The proliferation of the uteri (see

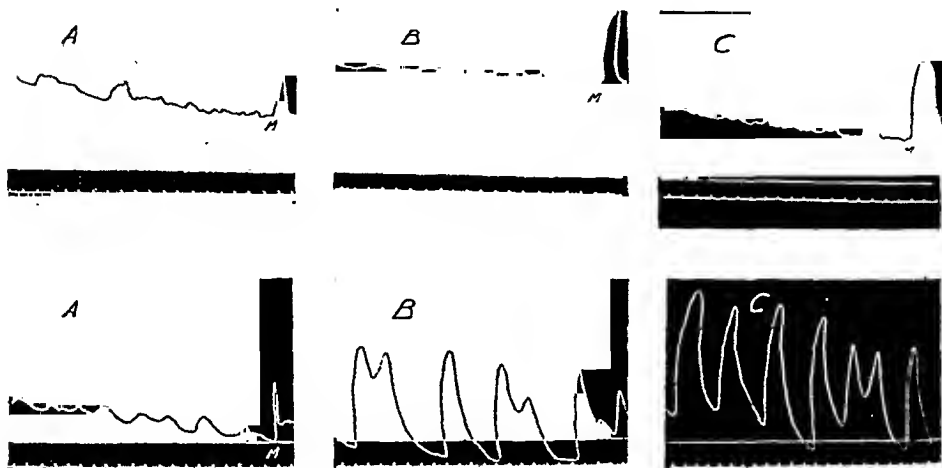


Fig. 2. Records showing inability of Theelin to induce uterine motility in the castrated rabbit after treatment with active progestin (top) and ability to do so after treatment with inactivated progestin (bottom). Top: A, motility after 5 days' treatment with 5 rabbit units of progestin. B and C, motility 12 and 24 hours respectively after 1000 r.u. Theelin per kilo (total, 2800 r.u.). Bottom: A, motility after 5 days' treatment with corpus luteum extract (inactivated by alkali) equivalent to 2.5 rabbit units of active progestin; B and C, motility 11 and 24 hours respectively after 5 r.u. Theelin per kilo (total, 15 r.u.). (Rabbits 11 and 34 respectively, table 2.) $\frac{1}{2}$ size. M, as before.

table 2) was not as great as would be obtained in standard test animals injected with similar doses of progestin. These doses (1.6–5.0 rabbit units) should produce complete proliferation in animals castrated 18 to 24 hours after mating and injected over the next 5 days. Such proliferation was not obtained in these animals probably because of the large amounts of Theelin injected on the 5th day. It has been adequately shown that oestrin has a detrimental effect on proliferation and if sufficiently large doses are given, proliferation even in the normal animal can be inhibited completely (Courrier, 1928; W. M. Allen, 1932; Leonard, Hisaw and Fevold, 1932).

TABLE 3

Effect on uterine motility of the daily administration of theelin (200 r.u. daily) during the first five days of pseudopregnancy in the rabbit

RABBIT	WEIGHT	MOTILITY POST COITUM					OVARY DESCRIPTION	ENDOMETRIAL PROLIFERATION
		MOTILITY ANTE COITUM	24 hours	48 hours	3 days	4 days	5 days	
14	1.9	+++ 60"-70"	++ irregular	++ irregular	0-+ irregular	0-+	0	7 corpora lutea ++
15	2.1	+++ 50"-60"	++ irregular	++ irregular	0	0	0	7 corpora lutea +
17	4.0	+++ 60"-70"	+++ 50"-60"	+++ irregular	0	0	0	8 corpora lutea 0
18	2.8	+++ 60"-70"	+++ 80"-90"	+++ irregular	+	0-+ irregular	+	10 corpora lutea +
19	2.8	+++ 60"-70"	+++ 40"-50"	+++ irregular	+	0	0	many corpora peritonitis
20	1.7	+++ 50"-60"	+++ 60"-80"	+++ irregular	0-+ irregular	0-+ irregular	0-+ irregular	7 corpora lutea +
21	2.5	++ 40"	+++ 50"	+++ irregular	0	0	0	9 corpora lutea +

Controls. Effect on uterine motility of the daily administration of theelin (200 r.u. per day for 5 days) in the normal unmated rabbit

WEIGHT	BEFORE FIRST INJECTION	AFTER FIRST INJECTION					48 HOURS AFTER LAST INJECTION
		24 hours	48 hours	3 days	4 days	5 days	
29	2.1 +	++ 80"-100"	++-++-++ 40"-50"	++-++ 85"-90"	++-++ 50"-60"	++ *see below	+
30	2.8 ++ 45"-60"	++-++ 60"-70"	++-++ 60"-70"	++-++-++-++ irregular	++-++-++-++ 60"-80"	++-++-++-++ irregular	++-++-++-++ irregular
31	2.9 -	++ 20"-30"	++-++ irregular	++-++ 60"-70"	++-++-++-++ 60"-90"	++-++-++-++ 60"-90"	++-++-++-++ irregular
32	5.4 -	++-++-++-++ 45"	++-++-++-++ 45"-60"	++-++-++ 45"-60"	++-++-++ 60"	++-++-++ 55"-60"	++-++ 80"-90"

* This animal ovulated during latter part of course of injections.

Summary. Corpus luteum extracts containing progestin (4.8-5.0 rabbit units) will completely prevent any motility response in the castrate rabbit from the intravenous injection of 1000 rat units of Theelin. Extracts containing 1.6 rabbit units of progestin will prevent a motility response from 200 r.u. Theelin per kilo but they will not inhibit 500 r.u. per kilo. Extracts in which the progestin has been inactivated by alkali have no motility-inhibiting effect whatsoever, for a dose of 5 r.u. of Theelin per kilo brings about complete oestrous motility when injected into animals receiving corpus luteum extracts which contained 2.5 to 4.0 rabbit units of progestin before inactivation.

EFFECT OF THEELIN ON UTERINE MOTILITY OF PSEUDOPREGNANT RABBITS (table 3, fig. 3). It has been definitely shown by several investigators that injections of large amounts of oestrin during the first few days after mating will completely prevent the appearance of proliferation but at the same time the development of histologically normal corpora lutea is not prevented (Courrier, 1928; Leonard, Hisaw and Fevold, 1932; W. M. Allen, 1932). We have, therefore, the apparent paradox of histologically normal corpora lutea developing without a concomitant proliferation of the endometrium. This unusual finding gives a method whereby the inhibiting effect of the corpora lutea upon the motility of the uterus may be studied without the presence of proliferation; and at the same time, motility studies may yield direct evidence regarding the functional capacity of these young corpora. Consequently in this series of experiments rabbits were mated and then injected subcutaneously with 200 rat units of Theelin per day for the next five days. The motility of the uterus was recorded daily throughout the period of injections and autopsy was performed the day following the last injection.

The motility data of this series of experiments are interesting in view of the fact that the uterine motility did not persist beyond the first 24 hours (or slightly later in several cases) after ovulation. In other words, despite the absence of corpus luteum effect as determined by the absence of proliferation, there was nevertheless, apparent corpus luteum function as determined by its effect on uterine motility. That this inhibition of motility was due to the corpora lutea present and not to the injection of the Theelin, is shown by the fact that four control rabbits in which coitus was not permitted (and hence in whom corpora lutea were not present) the injection of 200 rat units of Theelin daily for five days was accompanied by marked motility throughout the period of injections.⁴ (One doe (no.

⁴ A point of difference between the motility in the rabbits of this series and the motility that normally occurs during a similar period of pseudopregnancy in untreated rabbits may be mentioned in passing. In these Theelin-injected rabbits motility persisted through the first twenty-four hours *post coitum*, whereas, normally in the absence of injections of oestrin, the uterus usually becomes quiescent within five to eight hours *post coitum*. (Reynolds and Friedman, 1930; Reynolds, 1932.)

29) of the control group showed feeble motility on the last day of the experiment. It was found that this doe had ovulated several days previously and that there were normal corpora lutea in the ovaries. This occurred despite the fact that complete isolation of the does was maintained, and can only be explained as an instance of spontaneous ovulation.)

The histological studies of the ovaries and uteri of this series of animals revealed that corpora lutea were present in all cases except the controls. These corpora appeared to be histologically normal but the uteri showed little or no proliferation. They are in complete agreement with the series described previously in detail by one of us (W. M. Allen, 1932a). It should be mentioned that no. 19 at autopsy showed an early peritonitis both in gross and upon microscopic study.

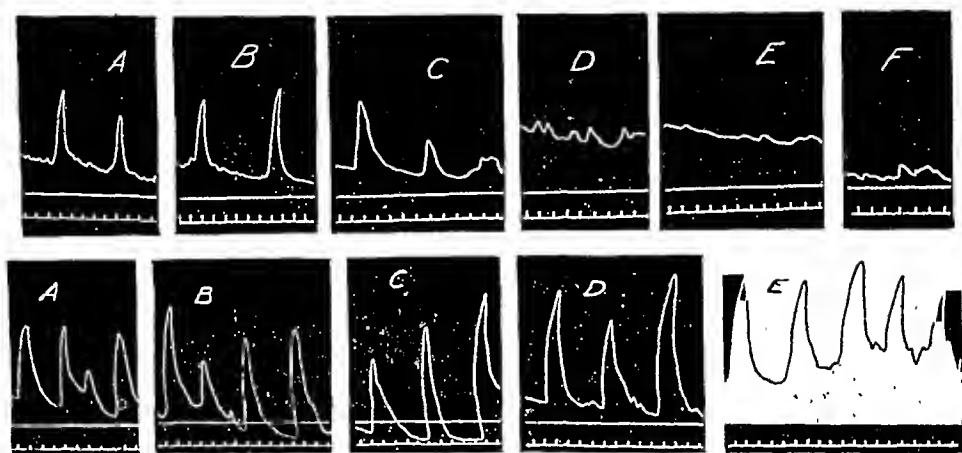


Fig. 3. Records showing uterine motility following coitus (and subsequent ovulation) in the presence of Theelin (200 r.u. daily for 5 days) (top) and uterine motility following injection of Theelin in the absence of ovulation (200 r.u. daily) (bottom). Top: A, normal motility before coitus; B, C, D, E, F, daily motility for first five days of pseudopregnancy in the presence of sufficient Theelin to prevent endometrial proliferation. Bottom: A, B, C, D, E, daily motility following injection of Theelin alone. (Rabbits 18 and 32 respectively, table 3.) $\frac{1}{2}$ size. *M* as before.

The immediate mechanism of this quiescence has not yet been explained, but a recent observation which Markee (1932) had occasion to make in a rabbit with a transplant of uterine tissue to the anterior chamber of the eye suggests that the motility changes we have recorded run *pari passu* with the vascular rhythmic contractions which he has described for the uterine mucosa. Markee found that the rhythmic vascular contractions cease with the vessels in dilatation, at seven hours after coitus. This is indeed a close correspondence in time with the effects we have observed in uterine motility, and probably represents a fundamental interdependence between the two phenomena. It may possibly be that the changes noted above are associated with the 10 to 20 per cent decrease in blood calcium which occurs several hours after coitus in the intact rabbit or after implantation of anterior hypophyseal tissue in intact or castrated rabbits (Hogben and Charles, 1932).

Summary. The subcutaneous injection of 200 r.u. of Theelin per day for the first five days after mating prevents the development of proliferation but does not maintain oestrous motility of the uterus. If, however, the same dose of Theelin is given to unmated rabbits (and, therefore, animals with no corpora lutea in their ovaries) normal oestrous motility is maintained.

DISCUSSION. The observation that daily injection of oestrin (200 r.u. daily for the first five days after mating) prevents the development of proliferation but does not affect the development of histologically normal corpora lutea brings up the question of whether or not the corpora are functional. The previous histological studies of one of us (W. M. Allen, 1932a) give no evidence regarding the activity of these corpora, since no proliferation was produced by them. However, the motility experiments which we have described above indicate directly that these corpora lutea are functional, even though they do not produce proliferation of the endometrium. The uterus becomes quiescent despite the daily injection of 200 r.u. of Theelin, only if corpora lutea are present (table 3). This might be taken as *prima facie* evidence of two hormones (i.e., one which causes proliferation and one which inhibits oestrous motility) since inhibition is obtained without the presence of proliferation. Such an assumption is not warranted, however, because it is quite possible that the daily injection of Theelin has so altered the endometrium that it is unable to become proliferated while under the action of the rabbit's own corpora. This in fact is so, for it has been definitely shown that sufficiently large doses of oestrin will completely inhibit the proliferative capacity of progestin-containing extracts (Tausk, de Fremery and Luchs, 1931; Leonard, Hisaw and Fevold, 1932; W. M. Allen, 1932a). Courier (1931) also has produced evidence that these corpora are functional. He has found that the continued injection of oestrin for 8 to 10 days after mating is compatible with perivascular changes despite extensive degeneration of the endometrium (and absence of proliferation) provided that corpora are present. We must conclude, therefore, that the corpora lutea under the conditions of these experiments are functional in that they produce either directly or indirectly a hormone or hormones which prevent oestrous motility of the uterus, and which sensitize the endometrium so that perivascular stromal changes may be produced following trauma to the endometrium. They do not prove conclusively that they produce progestin, but it may be supposed that they do because of Courier's observation that the endometrium is sensitized (assuming that progestin is necessary for the production of the perivascular changes).

The experiments described above also show conclusively that progestin-containing extracts of the corpus luteum contain a substance which inhibits normal oestrous motility and which prevents oestrous motility following the intravenous injection of Theelin. These observations are in apparent

agreement with the extensive experiments of Knaus, who, by studying (Knaus, 1927) *in vitro* responses of the excised uterus to certain drugs, notably pituitrin, has shown that during pseudopregnancy (Knaus, 1930b) the uterus is completely refractory to pituitrin and that near the end of either of these periods this refractoriness disappears. He has demonstrated also that this refractoriness to pituitrin disappears about 24 hours after the excision of the corpora lutea (Knaus, 1930a), and that upon excision of the pregnant horn of a unilateral pregnancy in the latter part of pregnancy (17 days or later) Knaus finds the uterus responsive to pituitrin (1930c), and finally, that corpus luteum extracts (made by the method of Corner and Allen, 1929) bring about a similar refractoriness to pituitrin, even before progestational proliferation is produced. Because of the fact that he can detect this inhibition sooner than proliferation can be fully developed, he has proposed this as a test for progestin (Knaus 1930d, e; 1931). This inhibition to pituitrin appears to be specific, he finds, since the uteri *in vitro* are not refractory to adrenalin or quinine. His evidence indicates therefore that progestin may be the factor responsible for pituitrin inhibition.

A rather different result with this type of experiment has been forthcoming from the work of Siegmund (1930a, 1930b) and Siegmund and Kammerhuber (1931) who applied this technic to the uterus of the mouse, rat and guinea pig respectively. These investigators, working in the same laboratory with Knaus, confirm his findings for the rabbit, but not for these other animals, even though corpora lutea of the oestrus cycle or of pregnancy are present at the time of excision of the uterus. Moreover, Siegmund finds that the uteri of these animals are not affected, as regards the pituitrin response, after the administration of corpus luteum extracts given in oestrus, metoestrus, pregnancy or after castration (Siegmund, 1930c). These corpus luteum extracts were shown to be active by their action on the uterus of the rabbit, in which animal they were standardized according to the method of Knaus, mentioned above. Siegmund, in a separate paper (Siegmund, 1931) discusses the significance of these findings, and is inclined to regard the peculiar response in the rabbit as an attribute of the corpus luteum which is species-specific. Robson and Illingworth (1931), also using the excised uterus, have confirmed Knaus' original finding that corpus luteum extracts contained a pituitrin-inhibiting substance for the uterus of the rabbit, but they have also produced evidence which they interpret as indicating that there may be two hormones, one of which proliferates the endometrium and one which causes refractoriness to pituitrin. They found that some placental extracts (but not all) as well as fluid from an ovarian cyst brought about inhibition of a pituitrin response but did not produce proliferation. These extracts apparently were not assayed for their oestrin content and therefore they may have contained enough oestrin to make the detection of progestin (by the proliferation

reaction) impossible. Better evidence for two hormones is perhaps found in the work of Fevold and Hisaw (1932). They have found that their crystalline corporin in some few instances does not bring about inhibition of the pituitrin response of the excised rabbit's uterus, even though it does produce good proliferation.

We are unable at the present time to offer an adequate explanation of these contradictory findings, but two considerations at least throw some doubt upon their physiological significance. First, the pituitrin response is at best a doubtful criterion of functional activity of the uterus owing to the fact that neither pituitrin nor pitocin has a discernible effect upon the quiescent, non-gravid uterus *in situ* in the unanesthetized rabbit (Reynolds, 1930b). The other consideration is a general one emphasized by van Dyke, Bailey and Bucy (1930), who stress the fact that due regard is not usually given to the importance of the ionic balance of the perfusing medium before and during an experiment in which the excised uterus is employed. Since the publication of their paper this aspect of *in vitro* work with the uterus has not been generally heeded with the meticulous care these investigators prescribe. We must conclude, therefore, that at the present time there is no conclusive evidence that there is a special hormone (differing from progesterin) which makes the uterus refractory either to pituitrin *in vitro* or to oestrin *in vivo*. Proof of two hormones can only be obtained by showing that chemically pure progesterin does or does not produce both reactions (i.e., proliferation and inhibition of oestrous motility) or that two fractions can be prepared, one of which produces one reaction and not the other, and *vice versa*.

Very little can be said regarding the chemistry of the hormone responsible for inhibition of motility *in vivo*. The fact that the extracts which we have used contain such a substance gives practically no evidence about the chemistry of the hormone, because the discarded fractions were not assayed for their inhibiting power. It is only by assaying all fractions that chemical evidence is attained. It is certain, however, that the active principle is destroyed by treatment with alcoholic potassium hydroxide (2 per cent KOH in 95 per cent alcohol). In this respect the hormone is chemically similar to progesterin. If one assumes that the discarded fractions were inactive, then most of the findings known regarding the chemistry of progesterin are also applicable to the chemistry of the uterine motility inhibiting hormone, and thus our experiments would appear to favor the view that a single hormone may be involved in these several physiological responses. The special chemical properties of progesterin have been discussed recently by one of us (W. M. Allen, 1932b).

SUMMARY

Progesterin-containing extracts of the corpora lutea of swine inhibit oestrous motility and prevent motility of the uterus following the intra-

venous injection of Theelin. The hormone responsible for this inhibition is chemically similar to, if not identical with, progesterin, in that it is destroyed by alcoholic potassium hydroxide.

BIBLIOGRAPHY

- ALLEN, W. M. 1930a. This Journal, xcii, 174.
1930b. This Journal, xcii, 612.
1932a. This Journal, c, 650.
1932b. Journ. Biol. Chem., (in press).
- ALLEN, W. M. AND G. W. CORNER. 1929. This Journal, lxxxviii, 340.
1930. Proc. Soc. Exp. Biol. Med., xxvii, 403.
- BUTENANDT, A. 1929. Deutsch. Med. Wochenschr., lv, 2171.
- CORNER, G. W. 1928. This Journal, lxxxvi, 74.
- CORNER, G. W. AND W. M. ALLEN. 1929. This Journal, lxxxviii, 326.
- COURRIER, R. 1928. C. R. Soc. de Biol., xcix, 224.
1931. C. R. de l'Assoc. des Anat., vingt sixieme Reunion 1.
- FEVOLD, H. L. AND F. L. HISAW. 1932. Proc. Soc. Exp. Biol. Med., xxix, 620.
- GOLDSTEIN, L. A. AND A. J. TATELBAUM. 1929. This Journal, xci, 14.
- HOGGEN, L. AND E. CHARLES. 1932. Journ. Exp. Biol., ix, 139.
- KNAUS, H. 1927. Arch. f. Exp. Path. u. Pharm., cxxiv, 152.
1929. Arch. f. Gynäk., cxxxviii, 201.
1930a. Arch. f. Gynäk., cxl, 181.
1930b. Arch. f. Gynäk., cxli, 374.
1930c. Arch. f. Gynäk., cxli, 395.
1930d. Arch. f. Exp. Path. u. Pharm., cli, 371.
1930e. Klin. Wochenschr., ix, 839.
1931. Klin. Wochenschr., x, 742.
- LAQUEUR, E., E. DINGEMANSE AND S. KOBER. 1930. Nature, cxxv, 90.
- LEONARD, S. L., F. L. HISAW AND H. L. FEVOLD. 1932. This Journal, c, 111.
- MARKEE, J. E. 1932. This Journal, c, 374.
- MARRIAN, G. F. 1932. Biochem. Journ., xxvi, 25.
- MEYER, R. K. AND W. M. ALLEN. 1932. Science, lxxv, 111.
- REYNOLDS, S. R. M. 1930. This Journal, xcii, 430.
1931a. This Journal, xcvi, 706.
1931b. This Journal, xcvi, 230.
1932. This Journal, c, 545.
- REYNOLDS, S. R. M. AND M. H. FRIEDMAN. 1930a. This Journal, xciv, 696.
1930b. This Journal, xciv, 705.
- ROBSON, J. M. AND R. E. ILLINGWORTH. 1931. Quart. Journ. Exp. Physiol., xxi, 93.
- SIEGMUND, H. 1930a. Arch. f. Gynäk., cxl, 573.
1930b. Arch. f. Gynäk., cxl, 582.
1930c. Arch. f. Gynäk., cxlii, 703.
1931. Arch. f. Gynäk., cxlv, 512.
- SIEGMUND, H. AND F. KAMMERHUBER. 1931. Zentral. f. Gynäk., lv, 521.
- TAUSK, M., P. DE FREMERY AND A. LUCHS. 1931. Acta Brev. Neerl. de Physiol., Pharm., Microbiol., i, 4.
- VAN DYKE, H. B., P. BAILEY AND P. BUCY. 1929. Journ. Pharm. Exp. Therap., xxxvi, 595.
- VELER, C. D., S. A. THAYER AND E. A. DOISY. 1930. Journ. Biol. Chem., lxxxvii, 357.

THE HIGH FREQUENCY RESISTANCE OF HUMAN TISSUE

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The extensive use of high frequency currents for heating the deeper tissues of the human body has made it desirable to obtain more information on the path of the current between the electrodes and the distribution of heat in the tissues. The factors which control the current distribution are the relative amounts and positions of the tissues and their specific resistance to the high frequency diathermy current. The earlier work of Nesper (1910) and the more recent work of Philipppson (1920) and Hemingway (1931) has shown that the impedance of tissue to alternating current decreases with frequency until high frequencies of diathermy are reached when the impedance to alternating current becomes a pure resistance. At these frequencies the alternating current traversing the tissue will follow the path of the lowest electrical resistance, when possible, and will avoid tissue of a high resistance.

In the measurement of the high frequency electrical resistance of tissues, it is necessary to use alternating current of a frequency of the same order of magnitude as the diathermy frequency, i.e., 500 to 3000 kilocycles per second. With lower frequencies the apparent resistance increases. For this problem we have used a high frequency Wheatstone bridge described in an earlier paper (Hemingway and McClendon, 1932). The alternating current used had a frequency of one million cycles per second and the tissue was balanced in the bridge circuit by means of a capacity in series with a high frequency resistor. It has been shown previously that tissue resistance measured in this manner gives the value of the true high frequency resistance from which the heat production in the tissue can be computed according to the Joule formula.

A possibility mentioned earlier by Wildermuth (1911) is that the high frequency resistance might change on removal of the tissue to the cell for measurement. It is known from the work of Herman (1872), Galeotti (1902), and others that the low frequency resistance (i.e., measured with alternating current of 1000 cycles per second) undergoes large changes on the death of the tissue. However, Hemingway and Collins (1932) have shown recently that although the low frequency resistance of voluntary

muscle undergoes wide variations as the tissue dies, the high frequency resistance remains unchanged for hours, providing the body temperature is maintained. This constancy of high frequency resistance of tissue on death is in agreement with observations of Bachem (1930).

Another possibility which arose was that the cooling of the tissue either after death or on transferring to a conductivity cell might change the high frequency resistance. To investigate this, electrodes were clamped onto a leg muscle of an anesthetized rabbit. The rabbit was placed in the air chamber enclosed by circulating water of any desired temperature mentioned in the earlier paper (Hemingway and Collins, 1932). The rabbit was killed, the body cooled with ice water circulating between the double walls of the chamber, and the temperature again brought back to body temperature. A thermocouple junction, placed on the electrode so as not to interfere with the resistance measurements, recorded the temperature.

TABLE 1
Variation of high frequency tissue resistance with temperature

TIME	TEMPERATURE	RESISTANCE
<i>hours</i>		<i>ohms</i>
0	32.6	59
0.5	32.6	59
3.0	21.6	73
7.5	19.2	85
9.5	33.4	56
10.0	32.2	60

The resistance was measured before death and at varying time intervals after death. The electrodes were not disturbed during the experiment. The results are given in the above table, where the elapsed time is measured from the death of the animal. The results prove that although the high frequency resistance of the tissue is increased by cooling, it returns to its former living value on again raising to body temperature. This makes it possible to obtain tissue from a fresh cadaver or from a surgical operation, raise the tissue temperature to body temperature in a conductivity cell, and obtain the true high frequency resistance of the living tissue.

Although it is possible to measure resistance changes due to death or temperature changes with an electrode clamped to living muscle of an animal, it is not possible to obtain specific resistance values of tissues for comparison, by this method, since the cell constant of the systems is not known. To measure specific values of the tissue resistance the conductivity cell, as shown in figure 1, was used. This consists of two telescoping glass tubes with a gold plated electrode at the closed end of the larger

glass tube and another gold plated electrode of the same size on the inserted end of the smaller tube. The tissue is completely enclosed and does not lose water by evaporation while in the bath. The cell constant is computed from the distance between the electrodes and their areas. The value of the specific resistance is

Specific resistance (ohms) = Measured H. F. resistance \times Electrode area (cm.) \div Distance between electrodes (cm.)

Fat, voluntary muscle, bone, and skin were removed from the living human body during surgical operation. Tissues from the internal organs were obtained from cadavers within two to three hours after death.

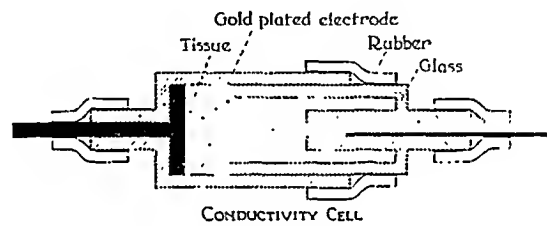


Fig. 1

TABLE 2

High frequency electrical resistance of human tissues (from surgical operations)

TISSUE	SPECIFIC RESISTANCE
	<i>ohms</i>
Skin.....	289
Fat.....	2,180
Bone.....	1,800
Muscle (voluntary).....	110

TABLE 3

High frequency electrical resistance of human tissues of internal organs (from fresh post-mortems)

TISSUE	SPECIFIC RESISTANCE
	<i>ohms</i>
Kidney.....	126
Liver.....	298
Heart.....	132
Spleen.....	256

The averaged results are given in the above tables and are in agreement with results of previous workers using other methods.

DISCUSSION. The relatively high skin temperature in diathermy may be attributed from these results and those of Bachem, to the layer of superficial fat of high resistance which the current must traverse. Bone, a high resistance tissue which can be avoided by the current, would receive little heat energy (except by heat conduction from other tissues). This is in agreement with the temperature distribution of diathermy current made by Schliephake (1929). Once through the skin and superficial fat, the current would follow the muscle tissue and blood wherever possible.

SUMMARY

The high frequency resistances of living human tissues have been measured with a high frequency Wheatstone bridge using alternating currents of one million cycles per second. Evidence is given to show that these values are the resistances of the tissues in situ. The high resistance of superficial fat and of bone explain the electrode heating in diathermy and the small heating of bone.

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BIBLIOGRAPHY

- BACHEM, A. 1930. Arch. Phys. Therap., X-Rays, Radium, xi, 391.
GALEOTTI, G. 1902. Zeitschr. f. Biol., xlv, 65.
HEMINGWAY, A. 1931. Radiology, xvii, 136.
HEMINGWAY, A. AND D. A. COLLINS. 1932. This Journal, xc, 338.
HEMINGWAY, A. AND J. F. McCLENDON. 1932. Physics, ii, 396.
HERMANN, L. 1872. Pflüger's Arch., v, 223.
NESPER, E. 1910. Physik. Zeitschr., xi, 20.
PHILIPPSON, M. 1920. Compt. rend. Soc. Biol., lxxxiii, 1399.
SCHLIEPHAKE, I. 1929. Zeitschr. f. d. ges. Exp. Med., lxvi, 212.
WILDERMUTH, F. 1911. Mitt. a. d. Grenzgeb. d. Med. u. Chir., xxii, 511.

THE INSENSIBLE WATER LOSS THROUGH THE SKIN

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According to the usual conception, water may be lost from the surface of the body either as the result of a physical process of diffusion, or as the result of an active secretion of sweat. “. . . as regards the first kind of loss by evaporation, the skin is apparently behaving as a semipermeable membrane, separating the blood plasma from the external surface of the skin. This membrane is permeable slowly by water, but practically impermeable by inorganic salts such as the sodium chlorid or bicarbonate of the blood plasma.” This is the view that Hancock, Whitehouse and Haldane (3) have adopted in their study of the loss of water and salts through the skin.

An examination of the literature indicates that the exact nature of the so-called physical process, generally referred to as insensible perspiration, is not so simple as is often assumed. There is no doubt that an insensible loss of water is constantly taking place from the skin under conditions in which the sweat glands are thought of as being quiescent. But whether this phenomenon is exclusively a function of the epidermal cells or whether it is really dependent upon the activity of the sweat glands is still obscure.

Jürgensen's (4) observations seem to indicate that the insensible perspiration is largely a phenomenon of the sweat glands. Thus he finds that while the sweat glands are in alternate periods of rest and activity, there are always some glands in activity even though their secretion may not be visible, and the skin feels dry and cool. As evidence of this unceasing activity on the part of the sweat glands, Cramer (1), and Schwenkenbecher and Spitta (14) point to the constant presence of salt on the body surface. As the epidermis is impermeable to sodium chlorid, it can come only from the gland orifices.

On the other hand, Loewy and Wechselmann (7) have made the interesting observation that individuals without sweat glands, when exposed in the resting condition to relatively low temperatures, lose as much water by way of the skin as normal persons, but not when either the heat production or

¹ The writer wishes to express her very great appreciation to Dr. A. L. Meyer for his interest and helpful direction during the course of this investigation.

the external temperature was raised. Then an undue elevation of the body temperature was observed. It is Loewy's (6) opinion that the insensible perspiration is determined in large measure by the temperature of the skin and its blood supply.

Other experimental evidence would lead one to suppose that both the sweat glands and the epidermal cells are responsible for the insensible perspiration, but that the elimination from the epidermis is not a passive or physical process, but a definitely controlled physiological process. This appears to be essentially the conclusion that Moog (8) has reached. Atropin, which inhibits the activity of the sweat glands, he finds, diminishes but does not abolish the insensible water loss from the skin. A venous stasis of short duration lowers the temperature of the skin and at the same time reduces the insensible output. On the other hand, a long continued venous stasis actually increases the output above the normal. That a cooling of the skin should be followed by an increase in water elimination can hardly be explained, according to Moog, on purely physical grounds, nor can it be accounted for by an edematous condition of the skin because Schwenkenbecher (13) and Moog (9) failed to establish a consistent increase in clinical cases of edema. Schwenkenbecher (11) attributes the increase under these circumstances to an accumulation of carbon dioxide, which appears to be a reasonable explanation, since it is easily demonstrated that carbon dioxide does stimulate the sweat glands. Moog further makes the rather unexpected observation that neither dermatitis nor the injection of histamine promotes insensible perspiration, although in each case the temperature of the skin as well as its blood supply increases. This, he asserts, is due to the fact that both inflammation and histamine have an injurious effect on the sweat glands.

Now the factors thus far considered, in connection with Moog's work, are conceived as affecting the insensible perspiration by their action on the sweat glands. It is to be noticed that the insensible loss of water is reduced through the agency of either atropin or histamine, but it does not entirely cease. That portion of the insensible moisture of the skin which continues to be eliminated in spite of an abeyance of glandular activity has its origin in the epidermal cells. But this loss, contrary to the usual assumption, is a manifestation of vital activity on the part of the cells themselves. If, for example, one paints the skin with formalin and thereby injures the sweat glands, the insensible perspiration not only continues, but actually increases. Apparently, then, the cells of the epidermis are capable of being stimulated to increased activity and must therefore be regarded as active agents in the phenomenon. Were the imperceptible loss from the epidermal cells a purely physical process, one would expect, says Moog, that as soon as the circulation stopped, it would show a sharp reduction. As a matter of fact, after death the loss of water shows only a

very gradual reduction which seems to be associated with the gradual death of the epidermal cells. It appears to be a part of Moog's conclusion that, although the two sources of insensible perspiration may function simultaneously, they are nevertheless quite independent.

Special staining methods enabled Unna (16) to demonstrate a system of minute canals in the dermis which had their origin at the apices of the papillae, and after spreading in radial fashion toward the surface layers, returned to the interpapillary region and finally ended in the ducts of the sweat glands. Obviously such an arrangement makes possible the evaporation of water at the orifices of the sweat glands which is not the product of an active secretion.

According to Frieboes (2) the passage of water through the epidermis is not wholly confined to the canalicular structure but is effected in large measure by a meshwork of fibers having hydrophilic properties. This is somewhat akin to the idea advanced by Rothman (10), namely, that during the process of keratinization water, which had previously constituted an integral part of the cell protoplasm as bound water or water of hydration, becomes free to undergo evaporation.

METHOD. *Phosphorus pentoxid method.* Anhydrous phosphoric acid (phosphorus pentoxid) is an excellent hygroscopic agent and was found eminently suitable for the absorption of water vapor from the skin. In order to give the powder greater surface, it was mixed with pumice previously heated to white heat by means of a blowpipe to remove any possible traces of organic matter. Two sets of U tubes were used. One collected water vapor from a given area of the skin at the same time that the other collected water vapor present in the room air. Each set of tubes was connected to a wet meter, and each meter connected to a negative pressure outlet by means of which the air flow could be regulated at will.

In determining the quantity of water vapor present in the room air, the air was drawn directly into the set of U tubes where it lost its moisture, and then through the meter where the volume of air was measured. In determining the quantity of water vapor given off by the skin, however, the room air was first allowed to pass through a glass applicator placed over a small area of the skin, and then into the U tubes containing the phosphorus pentoxid. The rubber tubes which carried the room air to both sets of U tubes were brought close together with adhesive tape in order that the air going to both sets of U tubes should be identical in composition. Thus, the difference between the quantity of water vapor collected over the skin in a given time (which also included the water vapor present in the room air), and the quantity of water vapor present in the same volume of room air, constitutes the amount of insensible perspiration that must have been given off by that particular area of the skin.

The same sets of U tubes could be used about forty times without being

changed. In the last experiment of one series in which the tubes had been used for thirty-nine experiments, the amount of moisture which passed over into the second tube of one set was 0.0009 gram, and of the other set 0.0006 gram. This gives some idea of the efficiency of the method. The glass applicator had a diameter of 5 cm. which means that it covered a skin area of approximately 19.6 sq. cm. Although the observations have been confined chiefly to two areas, namely, the left cheek and the left supra-mammillary region, they clearly indicate that the rate of insensible perspiration from these areas is definitely modified by certain changes in the physical condition of the air, and that the process is quite independent of any demonstrable activity on the part of the sweat glands.

It is possible that the quantity of vapor found might depend upon the rate of its removal from the enclosure. When the air is unsaturated, the evaporation of sweat, as is well known, is much more rapid in moving air than in still air. While it appears to be a characteristic of the insensible perspiration that the supply of water keeps pace with its evaporation, it is conceivable, nevertheless, that a slight excess of moisture might be present on the skin from time to time which would easily escape observation. The rate of air flow most commonly employed in these experiments lay between 0.3 and 0.5 cubic foot per half-hour. This means that the air of the enclosure was being changed at the rate of about three to five times per minute. Variations within these fairly wide limits had no measurable effect on the amount of moisture collected. When, however, the flow was definitely above these limits, e.g., 0.6 to 0.7 cubic foot per half-hour, there was a very noticeable increase in the elimination of moisture. Since the vapor tension of the room air as it enters the enclosure must vary inversely with the rate of air flow, and since the phenomenon that we are considering is one of evaporation which is determined by the vapor tension of the air, the insensible perspiration would be expected to bear a definite relationship to the current through the enclosure. As a matter of fact, there appears to be a critical velocity below which the elimination is practically independent of air velocity and above which the elimination increases with the rate of air flow. It is clear, however, that at the lower rates of air flow more water is available for evaporation than is actually evaporated.

Silver nitrate method. According to Schwenkenbecher (12), sodium chlorid is eliminated from the human skin exclusively through the activity of the sweat glands, and the epidermis is impermeable to an aqueous solution of sodium chlorid. It is generally held that sweat is a salt solution which is hypotonic with reference to the blood plasma (C. Kittsteiner, 5). If this is true, then the demonstration of salt on the skin surface may be accepted as evidence of activity on the part of the sweat glands. In order to avoid mistaking former activity for present activity, the area under observation was always freed from any possible traces of salt by gently

washing the area with distilled water a few minutes before the experiment began. The method of revealing the presence of salt is extremely simple. When the temperature of the room was sufficiently high to cause sweat secretion, a piece of smooth white paper applied firmly against the skin for a few moments would, upon being immersed in a ten per cent silver nitrate bath and then exposed to the sunlight, develop minute punctate areas of a deep brownish color. These punctiform areas indicate the position of active sweat glands and become more numerous as the temperature of the room rises. The method is sufficiently delicate to detect the presence of salt in a concentration as low as 0.005 per cent. Throughout this investigation there was not a single instance when evidence of insensible perspiration was not obtained, and only five instances of sweat secretion. In each of these five cases the elimination rose from 18 to 72 per cent above the average, and the silver nitrate test showed a positive reaction.

The experiments on the subject were made in the sitting position. All the experiments were carried out in a special room. When constant atmospheric conditions other than those naturally existing were required, air was supplied by a conditioning apparatus. Care was taken to place the applicator over the same skin area each time, and the silver nitrate test was applied immediately after each experiment.

EXPERIMENTAL RESULTS. *Effect of humidity.* If water appears as the result of diffusion through the skin acting as a semipermeable membrane, it must be present in the free state. Its evaporation would therefore be determined by the temperature of the skin and would continue until the air in contact with the skin became saturated. It is probable, however, that the process ceases some time before the saturation point is reached. This seems to follow from experiments with cobaltous chlorid. If discs of paper impregnated with cobaltous chlorid are placed within the enclosure, the amount of moisture absorbed falls considerably short of the amount collected under similar conditions by the phosphorus pentoxid method. At the end of a short period, say five minutes, the gain in weight of the cobaltous chlorid may agree very closely with the gain in weight of the phosphorus pentoxid, but at the end of half an hour the discrepancy becomes very great. This is not because the capacity of the paper is limited to the amount of water absorbed, for a considerably greater absorption will be found to have taken place if the paper be placed over water for half an hour, even though the water has a lower temperature than that which prevails in the enclosure. Now the aqueous tension of the hexahydrate of cobaltous chlorid has never been determined, but is probably of the magnitude of that shown by the hexahydrate of strontium chlorid or the pentahydrate of cupric sulphate. At 30°C. these salts exert a tension of about 12 mm. Hg as compared to 31.5 mm. Hg, the tension shown by water. The inference from these experiments appears to be that we are

dealing not with water having its origin in a process of diffusion, but with water of hydration.

This inference finds additional support in another group of experiments. If, instead of passing room air through the enclosure over the skin, we pass dry air, the mass of water vapor collected is always greatly increased, in some cases by as much as 100 per cent or more. This means that a relative humidity of approximately 20 per cent (one commonly employed in these experiments) suffices to reduce the vaporization 50 per cent or more. At this rate we would expect, in conformity with our previous argument, that

Effect of passing dry air—cheek

DATE (1931)	DRY BULB	WET BULB	REL. HUM.	EFF. TEMP.	VAP. TEN.	ELIMINATION	AIR VOL.
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Control

	°F.	°F.	per cent	°F.	inch/Hg	grams/hour	cu. ft./hr.
March 16	77.0	56.5	25.0	69.0	0.228	0.0782	0.930

After passing dry air

March 16	78.5	57.0	23.0	70.0	0.223	0.1536	0.908
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Control

March 31	77.5	56.5	25.0	69.3	0.237	0.0712	0.808
----------	------	------	------	------	-------	--------	-------

After passing dry air

March 31	78.0	56.5	23.0	69.6	0.219	0.1292	0.736
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Effect of passing dry air—chest

DATE (1931)	DRY BULB	WET BULB	REL. HUM.	EFF. TEMP.	VAP. TEN.	ELIMINATION	AIR VOL.
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Control

	°F.	°F.	per cent	°F.	inch/Hg	grams/hour	cu. ft./hr.
March 6	70.0	50.0	19.0	63.8	0.136	0.0268	0.690

After passing dry air

March 6	70.5	50.0	17.5	64.1	0.130	0.0556	0.812
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Control

March 17	72.5	53.0	23.5	65.8	0.187	0.0204	0.774
----------	------	------	------	------	-------	--------	-------

After passing dry air

March 17	73.0	53.0	22.0	66.1	0.180	0.0504	0.682
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the insensible perspiration would cease at a relative humidity of about 60 per cent. That this is actually the case was demonstrated by several experiments in which air at 70 per cent saturation was passed through the enclosure. Not only did vaporization stop, but the air itself on coming in contact with the skin lost moisture, indicating that a reversal of the process had taken place. (See figures on page 65.)

By way of further investigation of this particular point, the entire body instead of only a limited area of the body surface was exposed to gradually increasing relative humidities. It was now observed that when the humidity reached 60 to 70 per cent of saturation, the vaporization of insensible perspiration was diminished, and that a further rise in the relative humidity up to nearly 80 per cent actually caused a return of the amount of evaporated moisture to the normal level.

Effect of increasing relative humidity—check

DATE (1932)	DRY BULB	WET BULB	REL. HUM.	EFF. TEMP.	VAP. TEN.	ELIMINATION	AIR VOL.
	°F.	°F.	per cent	°F.	inch/Hg	grams/hour	cu. ft./hr.
Feb. 1	72.0	59.0	45.0	67.3	0.360	0.0668	0.852
Feb. 1	71.0	60.0	52.0	67.0	0.387	0.0658	0.872
Feb. 1	73.5	65.0	63.0	70.0	0.527	0.0570	0.820
Feb. 1	75.0	69.0	74.0	72.2	0.638	0.0580	0.832
Feb. 1	77.0	72.0	79.0	74.5	0.732	0.0800	0.832
Feb. 18	74.0	60.0	43.0	68.7	0.360	0.0798	0.824
Feb. 18	74.5	65.0	60.0	70.7	0.508	0.0630	0.848
Feb. 18	75.0	68.0	70.0	71.9	0.595	0.0604	0.844
Feb. 18	76.5	72.5	82.5	74.5	0.732	0.0726	0.854
Feb. 25	72.0	62.0	57.0	68.1	0.448	0.0606	0.844
Feb. 25	73.0	64.0	61.0	69.4	0.499	0.0644	0.826
Feb. 25	75.0	68.0	70.0	71.9	0.595	0.0558	0.810
Feb. 25	75.5	69.5	74.0	72.8	0.650	0.0784	0.812
Feb. 25	77.5	72.5	79.0	75.0	0.746	0.0778*	0.824
Mar. 3	73.0	63.0	57.0	69.0	0.465	0.0492	0.808
Mar. 3	74.0	66.0	65.0	70.7	0.555	0.0556	0.802
Mar. 3	75.0	68.5	72.0	72.0	0.616	0.0584	0.814
Mar. 3	78.0	73.5	81.0	75.8	0.783	0.0654	0.848
Mar. 3	78.0	74.5	85.0	76.1	0.810	0.0636	0.828

* Silver nitrate test showed sweating.

DISCUSSION. The present research was undertaken primarily for the purpose of throwing additional light on the nature of the process of insensible perspiration. The literature thus far has not made it clear whether this process occurs independently of the sweat glands or whether it really constitutes another phase of their secretory function. If the process is one

occurring quite apart from any activity on the part of the sweat glands, there still remains the question whether it is simply a matter of physical diffusion, governed solely by environmental factors, or whether it is an active phenomenon under physiological control.

During the course of this investigation numerous instances of the insensible loss of water from the skin have been observed in which the silver nitrate method failed to reveal the presence of salt. Unless the sweat glands are regarded as being capable of secreting a fluid entirely devoid of salt or containing salt in mere traces, these instances afford definite evidence that water having its source in the epidermal cells and not in the sweat glands is constantly being lost by evaporation. The attempt which is sometimes made to base such an opinion upon the fact that persons born without sweat glands may, nevertheless, lose as much water from the body surface as normal persons under ordinary temperature conditions, is hardly beyond criticism, for it is perfectly conceivable that an abnormality in respect to the sweat glands may involve an abnormality in respect to the insensible perspiration also. In other words, the capacity of losing water in the absence of sweat glands may have resulted as a compensation and may not exist in the normal individual.

If the insensible perspiration be regarded as a phenomenon unconnected with the sweat gland function, one may think of it as a physical process in which the skin, serving as a semipermeable membrane, permits the outward diffusion of pure or nearly pure water. According to this view, which is the one accepted by Hancock, Whitehouse and Haldane (3), the loss of water by evaporation would be expected to vary in a manner determined by the operation of certain simple physical laws. Some of the results of the present investigation are consistent with such a theory. This is true, for instance, of the effect of changes in atmospheric temperature. It also appears to be true of the effect of air currents when acting upon the body as a whole. However, when the influence of humidity is considered, the physical theory in this particular form does not suffice to explain the phenomena actually observed. The exposure of a relatively small area of skin to perfectly dry air causes the loss of water by evaporation to increase 100 per cent or more above the value found when the air has a relative humidity of only 20 per cent. When the same area is brought in contact with air having a relative humidity of approximately 70 per cent, evaporation ceases. Such a result is not consistent with the idea that the skin acts as a semipermeable membrane, and leads one to suspect that water is not present in the free state, but rather in a state in which its tension is considerably less than that of free water.

But there is still another peculiarity in the behavior of the insensible perspiration with respect to atmospheric humidity and one which makes it very doubtful whether we are dealing with a process essentially physical

in nature at all. In general, the initial effect of increasing the water vapor content of the air of a room, at temperatures which do not produce sweating, is to diminish the amount of evaporation from the skin; but with a still further increase in the water vapor content of the air, the evaporation from the skin begins to increase and returns finally to the normal level or may in fact exceed this level. The implication of this result appears to be that the skin is not acting as a physical entity, but rather as a vital structure possessing the inherent capacity of increasing its water output in spite of a gradually increasing humidity of the surrounding air. It may be of interest to remark incidentally that the beginning of this upward trend in the elimination was associated with the onset of an uncomfortable subjective sense of warmth.

By way of criticism it may be argued that owing to the difficulty of evaporation from the general body surface when the entire body is subjected to an increasing atmospheric humidity, the small area immediately under observation becomes richer in water either as the result of diffusion from adjacent areas or as the result of a local change in the state of the capillaries; and, since the movement of air through the applicator would favor an escape of moisture, any local increase in insensible perspiration could be attributed to the experimental conditions and would therefore not necessarily have any real physiological significance. As a matter of fact, however, the rate of air change in the applicator is no greater and probably considerably less than the rate of air change between the skin and clothing due to convection currents. It will also be recalled that there is always more water available for evaporation in a given area of skin than is actually evaporated. It is not easy to understand how, under such circumstances, any additional water could possibly result in an increased vaporization. That it is due to an increase in atmospheric temperature is hardly likely because an increase of 5°F. in the room temperature would be expected to increase the skin temperature relatively, but slightly. The vapor tension of the water in the skin would also increase slightly. But the vapor tension of the room air, by increasing the relative humidity to 80 per cent, has increased out of all proportion to the increase in vapor tension of the water in the skin. In spite of this the skin continues to increase its water output against a greatly increased vapor tension of the surrounding air. In view of the observation that an increasing humidification of the atmosphere does tend to diminish the insensible perspiration as measured locally, but that this initial effect is followed by a definite increase and that this appears to be coincident with the onset of discomfort, the evidence strongly suggests an active intervention on the part of a structure constituting the immediate source of the water undergoing vaporization.

We seem to be led to the following position. The water which by its evaporation constitutes the insensible perspiration is bound in such a

manner as to materially reduce its tension below that of water in the free state; but the water thus bound may under certain circumstances be released by the exercise of a vital function on the part of the binding structure. The exact locus of this physiological activity cannot be said to have been determined with certainty. Since this activity occurs without the demonstrable presence of sweat, one is strongly tempted to believe that it takes place in the epidermal cells or structures in the epidermis other than the sweat glands, although the possibility still exists that the sweat glands may secrete a fluid of very low salt content some time before the appearance of true sweat. But a possible activity of this sort could hardly be interpreted as secretory in nature because that would involve the evaporation of pure water.

SUMMARY

1. Throughout this investigation there was not a single instance when evidence of insensible perspiration was not obtained, and only five instances of sweat secretion, in each of which the elimination rose from 18 to 72 per cent above the average and the silver nitrate test showed a positive reaction, indicating that a totally different mechanism had come into play. It seems probable, therefore, that the two processes are quite independent.

2. This work has tended to show that water exists in the skin as bound water or water of hydration according to the theories of Frieboes and Rothman, and not as free water as must be inferred from the theory of Hancock, Whitehouse and Haldane.

3. The evidence also suggests that the process is really a vital one under definite physiological control when the effect of humidity on the entire body is considered. Then the skin acts as a vital structure possessing the inherent capacity of increasing its water output in spite of a gradually increasing humidity of the surrounding air.

BIBLIOGRAPHY

- (1) CRAMER, E. 1890. Arch. f. Hyg., x, 231.
- (2) FRIEBOES, W. 1922. Arch. f. Dermatol., cxl, 467.
- (3) HANCOCK, W., A. G. R. WHITEHOUSE AND J. S. HALDANE. Proc. Roy. Soc., Ser. B. cv, No. B: 734, 43.
- (4) JÜRGENSEN, E. 1924. Deutsch. Arch. klin. Med., cxliv, 193, 248.
- (5) KITTSTEINER, C. 1911. Arch. f. Hyg., lxxiii, 275.
- (6) LOEWY, A. 1914. Biochem. Zeitschr., lxvii, 243.
- (7) LOEWY, A. UND W. WECHSELMANN. Virchow's Arch., ccvi, 79.
- (8) MOOG, O. 1924. Zeitschr. exper. Med., xlii, 449.
- (9) MOOG, O. 1926. Verh. deutsch. Kongr. inn. Med., 299.
- (10) ROTHMAN, S. UND F. SCHAAF. 1929. Chemie des Verhornungsprozesses. Handbuch der Haut- und Geschlechtskrankheiten. 1 Bd. 2 Teil. Berlin: Julius Springer.

- (11) SCHWENKENBECHER, A. 1903. Deutsch. Arch. klin. Med., lxxix, 29.
- (12) SCHWENKENBECHER, A. 1925. Klin. Wochenschr., no. 5, 202.
- (13) SCHWENKENBECHER, A. 1926. Verh. deutsch. Kongr. inn. Med., 300.
- (14) SCHWENKENBECHER, A. UND O. SPITTA. 1907. Arch. f. exper. Path., lvi, 284.
- (15) UNNA, P. G. 1882. Kritisches und Historisches über die Lehre von der Schweiss-sekretion. Schmidt's Jahrb., 194, H. 1.

SIMULTANEOUS INTERNAL AND EXTERNAL STIMULATION OF THE IRIS BY ADRENIN

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The radial dilatation of the iris caused by adrenin, when the organ has been sensitized by removal of the superior cervical ganglion, has been studied under various conditions. The phenomenon appears after subcutaneous injection of adrenalin or its conjunctival instillation in rabbits (Meltzer and Meltzer-Auer, 1904) and after splanchnic stimulation or emotional excitement in cats (Elliott, 1912; Hartman, McCordock and Loder, 1923) or asphyxia (Kellaway, 1919). Under the same conditions it does not appear in the other eye, if that eye is left normally innervated. Both Elliott and Hartman and his collaborators noted a slight residuum of the phenomenon on exciting their animals when the adrenal glands were inactivated, a result which Cannon and Bacq (1931) attributed to the action of sympathin. It is clear that by proper sensitization of the iris an extra amount of circulating adrenin, whether injected or secreted, can have an effect which is not otherwise manifest. It seemed possible that by instillation of adrenalin into the conjunctival sac, the iris, though not obviously affected, might be sensitized, and that then a greater concentration of adrenin in the blood would become effective. If this should prove true, a simple method of studying the conditions which cause increased adrenal secretion would be available. For that reason the present study was undertaken.

METHOD. Cats were used. The superior cervical sympathetic ganglia were removed aseptically under ether anesthesia.

The observations were made in a dark room with the pupils illuminated by means of an electric lamp placed at a fixed distance (60 cm.) from the animal's eyes. The changes in the pupil and in the nictitating membrane were determined by direct measurement, made by holding a millimeter scale close in front of the eyes. The animals became accustomed to the procedure after some time. In standard conditions the readings were practically constant for each animal.

RESULTS. After the operation the animals were artificially angered (movements, showing a dog). Under these circumstances there appears

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a strong dilatation of the pupils which recedes sharply a few seconds after the end of the stimulation, leaving frequently a more moderate widening which disappears gradually after a variable time (1 to 3 minutes). This latter effect is more marked the stronger the emotional excitement. There occurs also a marked contraction of the nictitating membrane which lasts from 2 to 5 minutes.

During the first days (about 15) that follow the operation, if 2 to 4 drops of adrenalin 1:10,000 are instilled in one conjunctival sac (using the other side for control), there occurs immediately a contraction of the corresponding nictitating membrane, whereas there is no perceptible change in the pupil.

At about this time (15 days after the operation) a similar instillation of adrenalin begins to produce a dilatation of the pupil. As time passes this dilatation is earlier, more marked and longer.

When adrenalin is instilled in one eye and after 10 minutes or longer the animal is excited emotionally, there appears, as stated before, a dilatation of both pupils. The instilled one shows a more intense and lasting widening (5 to 10 minutes). If several of these emotional excitements are produced at short intervals (5 to 10 minutes) there occurs on the instilled side a much longer dilatation (3 to 4 hours) (see fig. 1, A).

Dilutions of adrenalin varying between 1:1,000 and 1:50,000 were used for the instillations. The stronger the solution, the earlier, the more marked and the more lasting the effects. All the results mentioned were obtained after using the 1:10,000 solution.

If the animal is emotionally excited some hours (3 to 8) after the instillation of adrenalin, when the effects of the instillation have apparently disappeared (both pupils having the same transverse diameter), there still occurs a difference in the consequent dilatation in favor of the instilled eye (see fig. 1, B). Removal of one adrenal and denervation of the other one (two cases) or removal of both adrenals (one case) diminish the intensity of all the above-mentioned phenomena but do not suppress them.

Intravenous injection of adrenalin, after instillation of adrenalin in one eye, produces a larger and more persistent pupillary widening in the instilled eye (see fig. 1, C).

Experiments were tried in normal cats without removal of the superior cervical ganglia, using doses of adrenalin similar to those mentioned above, and the results were negative. However, if strong solutions are instilled for a sufficiently long time (15 to 30 minutes) some effect appears (dilatation or contraction).

DISCUSSION. When the superior cervical sympathetic is removed, the iris is still innervated by the third cranial nerve and, as Cannon (1915) remarked, the instantaneous dilatation of the denervated pupil in emotional excitement is explicable as due to central inhibition of the tonically active constrictor impulses.

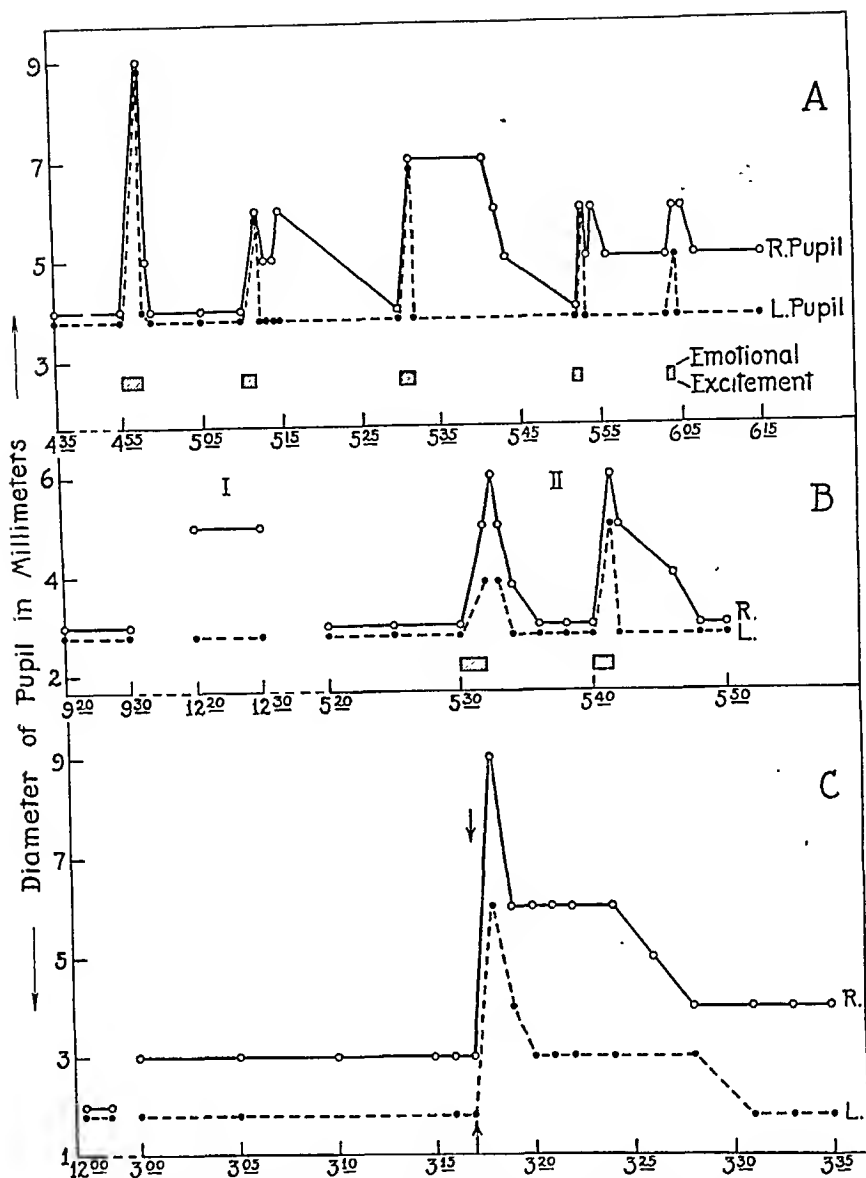


Fig. 1. Cats, with both superior cervical sympathetic ganglia removed. Continuous lines show the diameter of the right pupil, dash lines the diameter of the left pupil.

A. Adrenalin 1:10,000 instilled in right eye at 4:35. At 4:55, the animal was excited emotionally and there appeared a dilatation of both pupils, after which they recovered their previous dimensions. At 5:12 the animal was excited again. In the non-instilled eye the pupil recovered almost instantly, whereas the instilled one showed a more intense and lasting widening. At 5:30 another emotional excitement caused the same effect. At 5:52, after repeated production of emotional excitement, the pupil in the instilled eye was dilated for a few hours.

B. Adrenalin 1:10,000 instilled in right eye at 9:30. I shows the effect three hours after the instillation. II shows the different response of the instilled eye to emotional excitement after the apparent effect of the adrenalin had disappeared.

C. Right pupil is dilated from previous instillation of adrenalin at 12:05. At 3:19 an intravenous injection of adrenalin produced a much more marked and longer effect in the right pupil.

The smaller dilatation persisting after the emotional stimulation has ceased is due to adrenal secretion (Elliott and Kellaway, loc. cit.).

The negative effects on the iris on instillation of adrenalin during the first days after the denervation are due to lack of sensitization. At this stage the nictitating membrane already reacts.

The increased reactions of the pupil to emotional excitement after adrenalin has been instilled might be explained as a summation of effects. This hypothesis would also account for the more prolonged dilatation obtained in the instilled eye after repeated emotional excitement. This explanation, however, is apparently not admissible since it does not cover the more marked widening obtained several hours after the effect of the instillation has ceased and the pupils are equal (fig. 1, B). The great instability of adrenalin, especially when in contact with the tissues, is likewise not compatible with the idea of a slow absorption. Furthermore, the amount of adrenalin instilled (two drops of a 1:10,000 solution) is probably too small to render possible its action several hours after the instillation.

The only other hypothesis satisfactorily accounting for the facts is a sensitization of the iris on instillation. This theory, first proposed by Stuber, Russmann and Pröbsting (1923) would account for all of our results.

SUMMARY

The reactions of the iris, denervated by removal of the superior cervical sympathetic ganglia, to adrenalin instilled in the conjunctival sac and to emotional excitement were studied in cats.

Instillation of adrenalin in the conjunctival sac begins to produce a marked dilatation of the corresponding pupil only about 15 days after the denervation. As time passes this dilatation occurs earlier, is more marked and lasts longer. Emotional excitement produces stronger and longer pupillary widening of the previously instilled eye (see fig. 1a). On repetition of emotional excitement the effects increase. These reactions occur even several hours (3 to 8) after the instillation, when both pupils are apparently in identical conditions (see fig. 1b).

Inactivation of the adrenals greatly diminishes but does not wholly suppress the above-mentioned results.

BIBLIOGRAPHY

- CANNON, W. B. 1915. Bodily changes in pain, hunger, fear and rage. New York.
 CANNON, W. B. AND Z. M. BACQ. 1931. This Journal, xevi, 392.
 ELLIOTT, T. R. 1912. Journ. Physiol., xlv, 394.
 HARTMAN, F. A., H. A. MCCORDOCK AND M. M. LODER. 1923. This Journal, lxiv, 1.
 KELLAWAY, C. H. 1919. Journ. Physiol., liii, 211.
 MELTZER, S. L. AND C. MELTZER-AUER. 1904. This Journal, xi, 28.
 NEWTON, H. F., R. L. ZWEMER AND W. B. CANNON. 1931. Ibid., xevi, 377.
 STUBER, D. A., A. RUSSMANN AND E. A. PRÖBSTING. 1923. Zeitschr. exp. Med., xxxii, 396.

FLEXOR RIGIDITY OF THE HIND LEGS AND PRIAPISM IN THE "SECONDARY" SPINAL PREPARATION OF THE MALE CAT

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In the course of recent experiments a spinal preparation with as little "shock" as possible was required. It occurred to us that this purpose might be attained by first decerebrating and then decapitating the animal.

Such a "secondary" decapitate preparation, as we shall call it for convenience, shows an entirely different pattern from that of the ordinary decapitate animal (Sherrington, 1909). In the latter preparation the muscles present no special distribution of "tone" and the limbs remain in every position given to them. In contrast to this picture, the secondary decapitate preparation exhibits strong, persistent, springlike *flexor rigidity* in the hindlimbs, usually associated with marked *priapism* in the male. As this syndrome has not yet been described, so far as we know, a brief report of these experiments may be presented.

There are a few statements in the literature concerning the effect of low spinal transection after decerebration on the flexion reflex of an isolated flexor muscle in the cat (Sherrington and Sowton; Sassa and Sherrington; Forbes, Cobb and Cattell; Forbes, Barbeau and Rice; Gerard and Forbes). All these investigators report that the flexion reflex in such a preparation is slightly augmented, but on the other hand characterized by a marked diminution or an abolition of after-discharge.

In view of these statements the sustained flexor pattern observed in the experiments of this series was the more striking to us.

METHOD. All experiments were performed on the cat.

Under profound anesthesia induced by ether, a tracheal cannula was inserted, both carotids were ligated and a string was passed around the vertebral column for later ligation of the vertebral arteries according to the method devised by Sherrington. The atlanto-occipital membrane was then exposed. After freely opening the cranial cavity and the dura mater an intercollicular transection of the brain-stem was performed with a blunt spatula across the opening in the bony tentorium. The whole brain in front of this transection was then removed. In some of the experiments to minimize hemorrhage the vertebral arteries were compressed immediately prior to the severance of the brain-stem.

TABLE I

Results of secondary spinal transection after primary decerebration

NUM- BER OF EXPERI- MENT	ONSET OF F. R.	FLEXOR RIGIDITY (F. R.)	PRIAPISM	REMARKS
1	Immediate	+	In the first 7 experiments no annota- tions about priapism	F. R. lasting only a few minutes
2	Immediate	++++		
3		—		
4	Immediate	+++		
5	Immediate	+		
6		—		
7	Immediate	+++		
8	Immediate	+++	++	F. R. present for 5½ hrs.
9	Immediate	++++	++	F. R. present for 4 hrs.
10	Immediate	++++	++	F. R. for 14 hrs.
11	Immediate	++++	+++	F. R. for 23 hrs.
13	Immediate	+++	++	
14	Immediate	++++	++++	Some adductor and flexor rigidity in frontlegs
17	Immediate	++++	++	
18	After 12'	++	++++	F. R. not typically springlike
19	Immediate	+++	+++	F. R. strong for 3 hrs., stronger in hindleg with stronger D. R.
20	Immediate	++++	++++	Maximal F. R. for 12 hrs.
21	After 20'	++++	++	Onset of F. R. probably delayed because condition of animal was rather poor immediately after decapitation
				F. R. for 9½ hrs.
22	Immediate	+++	+++	F. R. maximal in hindleg with stronger D. R.
23	Immediate	++++	+	F. R. abolished by section of pos- terior roots caudad of L. I.
24	After 1 hr. 20'	+++	+++	F. R. for 5 hrs. At first F. R. stronger in hindleg with <i>weaker</i> D. R. After 1 hr. 30' stronger in hindleg with <i>stronger</i> D. R. Augmentation of F. R. after tertiary spinal transection at L. I. Abolished after section of posterior roots from L. I. on downwards
28		—	—	After decapitation (6 mm. below calamus) persistence of D. R. in hindlegs for 4 minutes
29	After 1 hr.	++	—	
30	Immediate	++	++++	F. R. stronger in hindleg with stronger D. R. Augmentation of F. R. after tertiary transec- tion at L. I.

TABLE 1—Continued

NUM- BER OF EXPERI- MENT	ONSET OF F. R.	FLEXOR RIGIDITY (F. R.)	PRIAPISM	REMARKS
31	Immediate	++	++	F. R. stronger in hindleg with stronger D. R.
32	After 1 hr.	++	+	Condition of preparation rather poor all through experiment. Tertiary spinal transection at L. I. did not result in augmentation of F. R.
33	Immediate	++	++	F. R. for 7 hrs. Stronger in hindleg with stronger D. R. Very strong F. R. and maximal priapism after tertiary spinal transection at L. I.
34	Immediate	+++	+	F. R. for 10 hrs. Augmentation of F. R. after tertiary spinal transection at L. I.; abolished after section of posterior roots
35	Immediate	++	++	Augmentation of F.R. after tertiary spinal transection at L. I. Abolished by section of posterior roots.
36	Immediate	+++	++	Increase of F. R. after transection at L. I.
38	Immediate	++	+	Augmentation of F. R. and priapism after spinal transection at L. I.
47	Immediate	++	+	Increase of F. R. and priapism after spinal transection at L. I.
65	Immediate	+++	—	F. R. for at least 7 hrs. Augmentation of F. R. after tertiary spinal transection at L. I.
67	Immediate	++	+	Slight adductor tone in frontlegs, appearing 5 hrs. after decapitation. F. R. increased after tertiary spinal transection at L. I. F. R. observed for 12 hrs.
(73)	Immediate	++	+	Secondary section at L. I. Increased D. R. of front legs. Decrease of F. R. in right leg after skinning. F. R. observed for 6 hrs.
(74)	Immediate	+++	+	Secondary section at L. I. Immediate increase of D. R. of front legs. F. R. observed for 9 hrs. Decrease in F. R. of right leg after skinning. Partial recovery after a few hours

TABLE 1—*Concluded*

NUM- BER OF EXPERI- MENT	ONSET OF F. R.	FLEXOR RIGIDITY (F. R.)	PRIAPISM	REMARKS
75	Immediate	+++	+	<i>Left adrenalectomy. Increase in F. R. of right leg after tertiary spinal transection</i>
76	98'	++	++++	<i>Bilateral adrenalectomy</i>
77	80'	++	++++	<i>Bilateral adrenalectomy</i>
78	8'	++	++++	<i>Bilateral adrenalectomy</i>
79	Immediate	++	++++	<i>Bilateral adrenalectomy after brain sections</i>
85	Immediate	++	++	<i>F. R. observed for 4 hrs. Animal sacrificed</i>
86	Immediate	++	++++	<i>F. R. observed for 4.5 hrs. Animal sacrificed</i>
87	Immediate	++	++++	<i>F. R. observed for 3 hrs. Animal sacrificed</i>

When decerebrate rigidity had appeared in the front legs or in all four limbs, the string around the vertebrals was pulled tight, the atlanto-occipital membrane was incised and the "secondary" decapitation performed. The level of this latter transection varied somewhat in the different experiments, the plane of the section being usually from 1 to 5 mm. caudal of the calamus scriptorius. Artificial ventilation and heating were started after the decerebration. In the experiments with secondary low spinal transection this operation was performed at the level of the first lumbar segment.

RESULTS. Such a secondary decapitation (see table 1) results not only in the disappearance of decerebrate rigidity, as might be expected since the primary decapitate preparation does not show this rigidity, but in the replacement of this extensor tone by a marked, sustained flexion posture, flexor rigidity (F. R.) of the hindlegs. This phenomenon usually appears immediately after the second transection, sometimes following an interval varying from a few minutes to a few hours. Apparently this latency in appearance of F. R. is short when the condition of the animal is good, as may be evaluated from the prompt establishment of strong decerebrate rigidity after the decerebration. If the onset of this extensor tone is delayed for some reason or another (too deep anesthesia, loss of blood, etc.) the F. R. sets in after a longer interval and may be only moderately developed.

With well established flexor rigidity, strong force must be exerted to overcome the flexion of these limbs, and, as soon as they are released, the

hindlegs return into flexion with a quick, springlike movement (fig. 1). In preparations with marked F. R. the tension required to extend the legs may be from 1300 to 1500 grams or higher. In one animal a tension of 2500 grams was necessary to extend the leg.

It is, however, possible to overcome this tendency to flexion by continuing the extension of a hindleg for 10 to 15 seconds. If after such a sustained passive extension the leg is gently released, it may remain extended or slowly return to the flexed position. If the leg remains extended, a slight stimulus applied to it, however, is sufficient to evoke prompt and lasting flexion.

The flexion is most marked in the hip and knee joints, less in the ankle joints, although in some instances marked dorsiflexion of the feet was observed. The superficial flexor muscles of the hip on the lateral side of the thigh, especially tensor fasciae femoris, and also the lateral knee flexors, may show fascicular twitchings, which often become more pronounced on passive extension of the leg.

The intensity of this F. R. is sometimes not equally strong in both hindlegs, but more pronounced on one side than the other. So far as our experience at present goes, we have nearly always observed that this occurs when the decerebrate rigidity after the primary decerebration was unequally distributed; the hindleg in which the stronger rigidity developed, always showed the stronger F. R. Only in one preparation the F. R. at first was stronger in the leg in which the decerebrate rigidity was less pronounced, although also here after one hour the F. R. became more marked in the limb with the stronger decerebrate rigidity.

Fig. 1 is part of a motion picture of a secondary decapitate cat in prone position on the operation table.

In pictures 1, 2, 3, 4, 5, 6 the right hindleg of the animal is extended by the observer. Between 6 and 7 it is released and snaps back into flexion.

The flexed position is reached in picture 10, showing that this flexion from the extended position occurs in about $\frac{1}{4}$ second.



Fig. 1

Usually the knee reflexes can be easily obtained; sometimes, however, we had the impression that the contraction of the flexor muscles was so strong that it interfered with the eliciting of these reflexes. Occasionally, with moderate intensity of the F. R., weak homolateral flexion and contralateral extension reflexes can be elicited, superimposed upon this persistent flexion.

The consequences of the secondary decapitation on the front legs are less striking. In the great majority of cases decerebrate rigidity disappears and the fore limbs become flaccid.

In a few animals a slight amount of decerebrate rigidity persisted in the fore limbs after a decapitation, about 5 mm. caudal of the calamus. This has already been observed by Magnus (1926), who states that only after



Fig. 2. Flexor rigidity and priapism in the "secondary" spinal cat (male).

decapitation about 11 mm. below the calamus decerebrate rigidity is completely abolished in the fore limbs in all cases.

Often, however, after a variable period ranging from one-fourth of an hour to a few hours, there appeared in the fore limbs a definite degree of contraction in the adductor muscles of the shoulder girdles and the flexors of the elbows.

Another feature of the picture which the secondary decapitate preparation presents is the appearance of marked *priapism* in the male cat. Often the erection of the penis is maximal, sometimes less pronounced (fig. 2). Dorsiflexion of the tail often elicits an augmentation of the erection, together with quick twitchings in the anal and perineal musculature as well as an increase of the flexion of the hind legs (see fig. 2). When priapism is well marked, dorsiflexion of the tail or tapping of the back of the animal

often provokes the ejection of a few small jets of urine or ejaculation of semen.

It is noteworthy that maximal priapism was observed in two distinct periods, namely, in most of the first 30 experiments during January and February, and in those performed after April 13. Priapism was much less pronounced and frequently absent in the experiments done during March and the first week of April. This is perhaps indicative of the presence of a seasonal factor in regard to this symptom; in this connection the statement in Brehm's *Tierleben* (1915) that the housecat gives birth to kittens during two periods, the first during April/May, and one during the beginning of August is of interest. Since the duration of pregnancy in the cat is 8 weeks, copulation would be most frequent during February and late spring.

Section of the posterior roots of the lumbosacral cord on one side immediately abolishes this flexor rigidity in the homolateral hind leg. Skinning of a hind leg results at first in a marked depression of the F. R. in that limb; after a few hours the rigidity may return to a certain extent, though we have not seen it attain its original value or the intensity of that in the contralateral hind leg.

It is significant that lifting the hindquarters of the preparation from the table by the tail, or placing the animal in the supine position results in a disappearance of the F. R. As soon as the animal is laid down again on the table, the F. R. reappears and the hindlimbs assume their typical position. Imitation of the stimuli which elicit the F. R. by rubbing the skin of the abdomen and of the inner aspect of the thighs and abduction of the legs, while the hindquarters of the animal are held lifted from the table, is followed by a definite reappearance of F. R. However, this F. R. is not as strong as that evoked by the contact with the table. Stretching the hindlegs when the animal is lifted from the table or is in the supine position does not elicit F. R.

Strong nociceptive stimuli applied to the erected penis, such as pinching this organ with an iris-forcep, often result in a temporary diminution or loss of the erection.

If the F. R. is only moderate in intensity, "tertiary" transection of the spinal cord at L. I. results in a marked augmentation of the F. R. In all cases in which this sequence of transections was followed, we have observed a maximal F. R. with strong dorsiflexion of the feet. As the participation of the ankle joints usually is least in the F. R. after secondary decapitation, the augmentation of the rigidity of their flexors is one of the most striking features after tertiary low spinal transection.

In a number of animals a low spinal transection at the level of L. I. was performed "secondarily." In these animals a marked or strong F. R. of the hindlegs also occurred. One of these showed the strongest F. R.

obtained in these experiments, as a tension of more than 2500 grams was required to extend the hindlegs.

In another series of 16 experiments (see table 2) we have investigated the significance of the time-interval between the primary decerebration and the secondary decapitation in the development of the syndrome described.

TABLE 2

Secondary decapitation after primary decerebration with various intervals between the two transections

NUMBER OF EXPERIMENT	INTERVAL BETWEEN TWO TRANSECTIONS	F. R.	ONSET OF F. R.	PRIAPISM	AUGMENTATION OF F. R. AFTER TERTIARY SPINAL TRANSECTION AT L. I.	REMARKS
53	14'	+++	Immediate	—	+	Castrated male
54	1 hr. 24'	—		—		
55	32'	++	Immediate	+	—	
57	40'	—		—	+	
58	10'	++	Immediate	++	+	
59	20'	+++	Immediate	—	+	
61	20'	++	Immediate	—	+	
62	30'	++	1 hr. 10'		+	Persistence of D. R. in both hind legs abolished after transection at C IV. F. R. after transection at L. I.
63	30'			—	+	
64	50'	—		—	—	Persistence of D. R. in right hind leg. Both hind legs flaccid after transection at C III. No F. R. after transection at L. I.
66	17'	++	2 hr.	+	+	F. R. for at least 12 hrs.
67	37'	++	Immediate	+	+	
69	40'	+	2 hr.	—	+	
70	50'	+++	Immediate	+	+	F. R. for 24 hrs.
71	1 hr.	+++	Immediate	+	+	F. R. for at least 8 hrs.
72	1 hr. 15'	+++	?	—	+	F. R. for at least 6 hrs.

From experiment 54, in which no F. R., nor priapism developed, it might seem as if in this respect a time factor was present. But experiments 71 and 72, in which a strong (+++), though not maximal (++++) F. R. set in, although there was an interval of 1 hour and 1 hour 15 minutes respectively between the decerebration and decapitation, show that this conclusion is not warranted. Perhaps prolonging the interval between these two transections results in a somewhat weaker F. R.

which occasionally sets in after a longer latency. The priapism was certainly much less pronounced than in experiments of table 1, but here the above mentioned seasonal factor may have entered, as these experiments were performed during the month of March. It would require special extensive investigation to settle this point.

The intensity of these phenomena may show slight fluctuations in the course of prolonged observation, but very often both the F. R. and priapism are very strong and even maximal over a period of many hours. This syndrome, flexor rigidity of the hindlegs associated with priapism, has been observed for as long as 26 hours. It seems as if such a secondary decapitate preparation is more resistant than the ordinary primary decapitate preparation, 24 hours being quite an unusually long time of survival for the latter.

In a few experiments secondary decapitation was performed in female cats. In these animals definite F. R. also appeared, but less marked than in the male cat.

DISCUSSION. The flexor rigidity described in these experiments is a reflex phenomenon, since it is abolished by section of the posterior roots of the lumbosacral cord. Its diminution after skinning of a hindleg demonstrates the operation of exteroceptive stimuli, whereas its abolition after section of the posterior roots proves the concomitant activity of proprioceptive impulses in this reflex pattern.

The fact that passive extension of a hindleg does not produce F. R. when the animal is lifted from the table or is in the supine position, shows that this rigidity is not evoked by the passive stretching of the flexor muscles by the observer in testing the F. R.

Since this is the only manipulation of the preparation during the observations, the F. R. is apparently elicited by exteroceptive and proprioceptive stimuli arising from the contact of the lower part of the abdomen and the ventral aspect of the legs and the posture of these limbs when the animal is in the prone position on the table.

The abduction and strong flexion of the hindlegs is part of the normal copulation posture of the male cat. This abduction of the legs, together with the sensory stimulation of the skin of the abdomen and the medial aspect of the thighs, are probably the imitation in these experiments of the adequate stimuli which are active in the male cat during normal copulation by the contact of these regions with the hind quarters of the female.

It is plausible to interpret the adduction of the shoulders and the flexion of the elbows often present in these animals as part of a grasping reflex which may be seen in the frontlegs of the male during coitus. Furthermore, the augmentation of the F. R. and priapism together with ejaculation which results from dorsiflexion of the tail is in agreement with the fact that during copulation the tail of the male cat assumes a similar posture.

There can be little doubt, therefore, that this syndrome is the manifestation of a sexual reflex pattern, released in the lumbo-sacral cord after secondary spinal transection.

Since this syndrome does not occur after primary decapitation its appearance can not be explained on the basis of severance of anatomical pathways descending from the cerebrum or brain stem. *Therefore, a functional factor must operate.*

It has been shown by Wachholder that during strong decerebrate rigidity action currents are not only present in the extensor muscles, as is long known, but also in the flexors. This author concludes that the extension of the limbs in decerebrate rigidity does not prove an unequal distribution of central innervation, but is simply due to the greater strength of the extensor muscles.

This statement is not beyond criticism, since it may well be that the action currents appearing in the flexors during strong decerebrate rigidity are only strong effects of the marked action currents in the extensor muscles. However, if Wachholder's conclusion is correct, it is conceivable that secondary spinal transection, which abolishes the extensor factor in decerebrate rigidity, allows the flexor mechanisms to emerge and so results in F. R. We have tested this possibility in the following way.

In a few animals we eliminated in one hindleg the activity of the extensors, first of the hip, by freeing these muscles from their points of insertion, and then of the knee by sectioning the femoral nerve. Upon decerebrating this preparation the three intact limbs showed the usual decerebrate rigidity, whereas the operated hindleg remained flaccid. Subsequent decapitation produced typical F. R. in both hindlegs. Thus, if concomitant activity of flexor muscles exists at all in decerebrate rigidity, it is not strong enough to cause flexion of a hindleg. Therefore, another functional factor must act.

This leads directly into a discussion of the still enigmatic problem of spinal shock. Sherrington, long ago, made the interesting observation that a second spinal transection performed after the "shock" following an initial low transection has disappeared does not result in a renewed depression of spinal activity.

The fundamental fact in our experiments is that marked flexor activity can be produced *immediately* after the secondary transection, showing absence of spinal shock as far as the flexor mechanisms are concerned.

Apparently by performing a secondary transection of the cord one eliminates, or greatly reduces, the time interval which exists for such a long time in the primary spinal preparation. Yet the question still remains why "shock" is absent or at least considerably less pronounced in the "secondary" spinal animal.

It is of interest that the blood pressure in such a preparation is just as

low as in a primary decapitate animal, showing that the spinal vasoconstrictor centres, in contrast to the flexor mechanisms, are in "shock" in the secondary decapitate preparation.

These experiments emphasize the general difference in distribution of extensor and flexor mechanisms in the neuraxis, although of course these two basic groups of reflexes are in the last analysis under the control of the entire central nervous system.

As is well known since the work of the schools of Goltz and Sherrington, some extensor activities are retained in the isolated spinal cord of the chronic preparation (homolateral extensor thrust and crossed extension reflex). Our experiments show that weak crossed extension reflexes can be obtained in the acute spinal animal against a background of moderate F. R. Such extensor activities, however, are meagre as compared with the mass of extensor reflexes of the brain-stem preparation (decerebrate rigidity and postural reflexes), indicating that undoubtedly the bulk of extensor mechanisms has shifted phylogenetically towards the brain-stem.

With regard to flexor reflexes it has long been known that they are the first to emerge in the later stages following spinal transection. The more recent observations mentioned in the introduction show that the flexor reflex may indeed be slightly augmented in the acute spinal preparation. These reactions represent primitive withdrawal responses to nociceptive stimuli mediated by the spinal cord. The experiments of this series strikingly demonstrate that under the special conditions of the acute secondary spinal preparation powerful, persistent flexor activity can be revealed which does not depend upon nociceptive stimuli, but is part of a sexual reflex pattern. Thus, important primitive flexor mechanisms, one for protection of the individual against noxious environmental influences, the other for the preservation of the species, are retained at spinal levels.

SUMMARY

1. In these experiments the results of a "secondary" spinal transection following "primary" decerebration of the animal (cat) are described.

2. In such an acute "secondary" spinal cat a syndrome appears which consists of strong springlike flexor rigidity (F. R.) in the hind legs associated with priapism.

3. This syndrome occurs in the secondary decapitate preparation as well as in the secondary low spinal preparation with transection of the spinal cord at L. I.

4. Section of the posterior roots on one side of the lumbosacral cord abolishes the F. R. in the homolateral hind leg, indicating that this F. R. is a reflex phenomenon.

5. Skinning of a hind leg after the establishment of F. R. results in a diminution of the F. R. Therefore exteroceptive impulses are involved.

6. From the observations under 4 and 5 it is apparent that proprioceptive impulses are also operating in the production of this F. R.

7. The F. R. disappears when the animal is lifted from the table or placed in the supine position showing that the adequate exteroceptive and proprioceptive stimuli initiating this reflex rigidity arise from the contact of the ventral aspect of the lower parts of the body with the table and the posture of the limbs in this prone position.

8. This syndrome is regarded as a copulation reflex pattern released in the lumbosacral cord after secondary spinal transection.

9. The absence or diminution of spinal shock as a possible explanation of this release is advanced.

10. The results of these experiments together with data from the literature indicate a general difference in the distribution of extensor and flexor mechanisms in the CNS.

BIBLIOGRAPHY

- BREHM. 1915. *Tierleben*. 4th ed., Mammals, Vol. iii.
FORBES, A., S. COBB AND H. CATTELL. 1923. *This Journal*, lxv, 30.
FORBES, A., A. BARBEAU AND L. H. RICE. 1927. *This Journal*, lxxxi, 476.
1931. *This Journal*, xcvi, 484.
GERARD, R. W. AND A. FORBES. 1928. *This Journal*, lxxxvi, 178.
MAGNUS, R. 1924. *Körperstellung*. Springer, Berlin.
SASSA, K. AND C. S. SHERRINGTON. 1921. *Proc. Royal Soc. London*, B. xcii, 108.
SHERRINGTON, C. S. 1909. *Journ. Physiol.*, xxxviii, 375.
SHERRINGTON, C. S. AND S. C. M. SOWTON. 1915. *Journ. Physiol.*, xlix, 331.
WACHHOLDER, K. 1928. *Pföger's Arch.*, ccxxi, 66.

RESPONSE OF THE NICTITATING MEMBRANE TO PROLONGED STIMULATION OF THE CERVICAL SYMPATHETIC

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The present research was undertaken in order to study fatigue phenomena in the cervical sympathetic, and especially in order to see whether or not there are differences in the fatigability of the pre- and post-ganglionic elements.

A survey of the literature has shown that whereas the problems of fatigue have been fairly thoroughly studied in the cerebro-spinal system, very little, comparatively, has been done on that subject in the autonomic nervous system. Eve (1896), trying to find the histological changes produced by fatigue in the cell bodies in the superior cervical ganglion, excited during long periods the pre-ganglionic fibers of the cervical sympathetic and found that at the end of the longest stimulation (12 hours), the vaso-constrictor apparatus of a rabbit's ear was quite capable of vigorous action. He also found evidence of an acid reaction in the nerve cells of the superior cervical ganglion when directly stimulated or when stimulated through the cervical sympathetic under impaired circulatory conditions.

Brodie and Halliburton (1902) excited the splenic nerve and recorded the contraction of the spleen. They found that very rapidly a condition appeared similar to fatigue of the end organ in the nerve-muscle preparation. Blocking the impulses by cold, thus preventing them from reaching the end organ, they demonstrated that even after six hours of excitation, the nerve is practically as excitable as it was at the start, for a good splenic contraction is obtained when the cold block is removed. They also found that the post-ganglionic fibers, after six hours of excitation, were still able to maintain constriction of the arterioles, although the constriction showed a gradual decrease. There is no statement in either of the above mentioned papers about the frequency of stimulation, a condition of primary importance, as we shall see later.

METHODS AND RESULTS. The experiments were performed on cats. The cervical sympathetic nerve was carefully dissected and separated from the vagus, 2 cm. before and immediately beyond the superior cervical

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ganglion, between it and the skull. Two electrodes for excitation, conveniently isolated, were placed on the nerve 2 or 3 cm. before the ganglion and another pair of electrodes was hooked on the trunk beyond the ganglion. Since the post-ganglionic range was short, we cannot be sure that the stimulus did not spread to the ganglion itself. To prevent possible spreading of the current to other structures *via* the vagus the latter was severed just above the ganglion, before entering the skull, and also below, opposite the electrode on the pre-ganglionic fibers. Most of the experiments were performed on the right side.

The contraction of the nictitating membrane was recorded by means of a common muscle lever with a 4- or 5-fold magnification.

A Harvard inductorium, arranged so as to use the minimal stimulus to evoke a maximal effect, was employed to produce the excitation. A



Fig. 1. Contraction of the nictitating membrane. I, pre-ganglionic stimulation; II, post-ganglionic stimulation (42 break induction shocks per second). Time, 5 seconds. In this and the other figures the drop in the lowest line marks the period of stimulation.

test showed that it delivered about 42 break shocks per second. A double-throw double-pole switch allowed an almost instantaneous change of the place of excitation without varying the strength of the current.

As the investigation proceeded, the mode of excitation was altered. In early experiments the pre-ganglionic fibers were stimulated until the record showed a progressive decrease in the contraction of the membrane. As this was interpreted as a sign of fatigue, the excitation was quickly changed to the post-ganglionic fibers. Immediately the record showed a vigorous contraction of the membrane which persisted at the same level during excitation for much longer periods than the pre-ganglionic. Figure 1 is a typical record of this reaction, found in many animals.

In another series of experiments I determined the general form of the curve when the excitation was maintained for a long time, first on the pre-ganglionic fibers and afterwards on the post-ganglionic. The results were

quite constant. Figure 2 illustrates one of these experiments. The lower record displays the effect of continued excitation of pre-ganglionic fibers, the upper record that of post-ganglionic fibers. While the former clearly

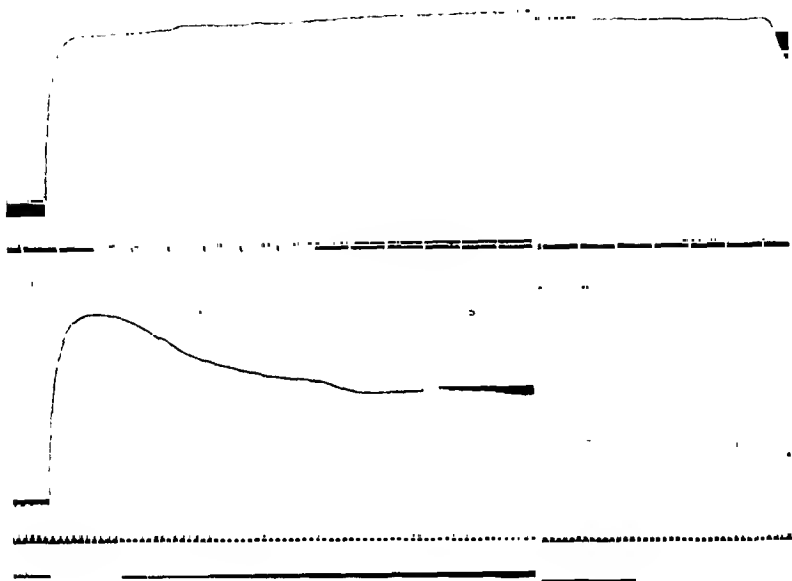


Fig. 2. Upper record: contraction of the nictitating membrane produced by prolonged post-ganglionic stimulation (42 break induction shocks per second). Time, 30 seconds. Lower record: contraction produced by pre-ganglionic stimulation (42 induction shocks per second). Time, 5 seconds. Between the two parts of the record there was an interval of 10 minutes.

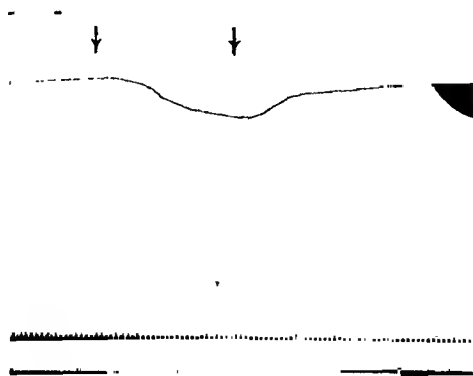


Fig. 3. Record showing the importance of the blood supply in maintaining the contraction of the nictitating membrane produced by prolonged post-ganglionic excitation. Between the arrows both carotid arteries were compressed.

shows signs of fatigue, the latter shows none. The speed of the drum was approximately the same in both cases. The time in the lower tracing was recorded at intervals of 5 seconds and in the upper at intervals of 30 seconds. The total time of excitation was about 20 minutes.

In a third series of experiments I studied the influence of the blood supply on contraction of the membrane during excitation of post-ganglionic fibers. Figure 3 is a record of one of these experiments. During the period between the arrows the carotid arteries on both sides were completely blocked. As the record shows, the membrane immediately relaxed somewhat but with no tendency to reach the base line. When the blood was allowed to flow again, the membrane promptly contracted to the previous level. That this is not a passive, mechanical effect is proved by the fact that cutting off the blood supply when the membrane is not contracted produces no change.

DISCUSSION. The peripheral neuromuscular mechanism involved in the contraction of the nictitating membrane includes the pre-ganglionic fibers, the post-ganglionic fibers, the connecting apparatus (ganglionic cells and synaptic junctions), and smooth muscle. Whenever the pre-ganglionic fibers are excited, the connecting apparatus is, of course, brought into play.

The experiments seem to show that while fatigue is rather readily produced by excitation of the pre-ganglionic fibers, it is much more difficult to provoke when post-ganglionic fibers are stimulated, even though the stimulation is prolonged. They also show the necessity of an adequate blood supply for the optimal function of the post-ganglionic system. These facts, however, should be carefully analyzed before drawing any conclusions.

Is it a real fatigue that happens as a consequence of the protracted pre-ganglionic excitation? The first possibility to consider is that the relaxation of the membrane in spite of the maintained stimulation is not a true fatigue, in the physiological meaning of the word, but the phenomenon analyzed by Howell, Budgett and Leonard (1894) as "stimulation fatigue," a term suggested by Gotch (1910) to indicate a loss of excitability at the point where the electrodes are applied. "Stimulation fatigue" was found to occur when sympathetic nerve trunks are excited during long intervals, especially when they are non-medullated, but without complete exclusion of the medullated fibers. If the electrodes are shifted peripherally along the nerve to a new place, the organ studied responds again with the same strength as before. Subsequently, Brodie and Halliburton (1902) confirmed these observations and showed also that when the nerve had lost its responsiveness at the points where the electrodes were located, it still was able to conduct impulses through that zone.

Apparently "stimulation fatigue" occurred to a certain extent in some of our experiments, because after a time shifting the electrodes along the

nerve in either direction produced a higher contraction; it did not, however, seem to be the predominant phenomenon. Indeed, the higher contractions thus evoked were neither equal in height to the first one nor did they last long. The two small increases shown in figure 1 during pre-ganglionic stimulation were caused by moving the electrodes to a new place.

In order to prove that "stimulation fatigue" is not an essential phenomenon and also in order to eliminate the more remote possibility of a functional fatigue along the nerve fibers, other experiments were performed in which the impulses were blocked before they reached the superior cervical ganglion, by means of a current of cold saline solution ($4^{\circ}\text{C}.$), bathing the nerve at a convenient distance from the exciting electrodes. The nerve was constantly excited, and every now and then the block was removed by changing the cold flow to a warm flow of the saline solution. Whenever the block was thus removed, the membrane contracted to the same height as before or higher. In this case the excitation was maintained during 50 minutes; after that the experiment was discontinued because it seemed demonstrative enough. These experiments show clearly that the signs of fatigue observed when the pre-ganglionic fibers of the cervical sympathetic are excited are not due to a loss of excitability of the nerve either at the point stimulated or along the fibers.

Another possibility is that the relaxation of the membrane is due to a process of inhibition similar to that described by Wedensky (cited by Ioteyko, 1904) in the nerve-muscle preparation and depending on the frequency of stimulation. He found that for maintaining a muscle in tetanus at a steady tension, the frequency of the stimulation had to be decreased as the excitation was prolonged. In other words, when a muscle begins to relax in spite of a continued stimulation, it can be brought to the initial level by diminishing the frequency of stimulation, showing that inhibition rather than fatigue is the cause of the phenomenon. Similar observations to those of Wedensky have been more recently reported by Davis and Davis (1932).

In order to test the possibility of a phenomenon of this type, the experiments were repeated, exciting with the same inductorium but regulating the number of induction shocks by means of an adjustable rotary interrupter. The pre-ganglionic fibers were excited first with a frequency of about 42 break induction shocks per second as in our previous experiments, and when the nictitating membrane began to relax, the rate of stimulation was changed to 3 shocks per second. As demonstrated in figure 4, which is a typical record obtained under these circumstances, the membrane contracts again to an appreciable extent and stays contracted as long as the excitation persists. The same result occurs when the second stimulation is applied with frequencies ranging from 3 up to about 20 shocks per second.

After these experiments, which strongly support the view that the fatigue-like phenomena observed are probably of the inhibitory Wedensky type, we excited the pre-ganglionic fibers at a frequency of about 10 shocks per second during one hour, with no evidence of fatigue. Thereafter the nerve was obviously injured at the site of stimulation, probably because of the type of electrodes used, and no reliable information could be obtained. Prolonged excitation of post-ganglionic fibers with a similar rate produced no signs of fatigue for periods as long as an hour and 15 minutes.

From the evidence gathered in our experiments we can safely conclude that no signs of fatigue in the physiological sense of the word are evident in the contraction of the nictitating membrane after periods of one hour of constant stimulation of either pre- or post-ganglionic fibers *when adequate frequencies of excitation are employed*. If frequencies above a certain critical level are used, fatigue-like phenomena occur, undoubtedly related

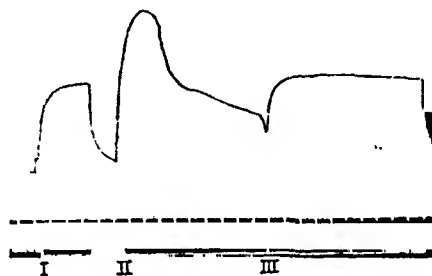


Fig. 4. Record illustrating the influence of the frequency of pre-ganglionic stimulation on the contraction of the nictitating membrane. I, stimulation at a rate of 3 induction shocks per second to test the effectiveness of the stimulus; II, prolonged stimulation at a rate of 42 shocks per second; III, 3 shocks per second.

to the Wedensky inhibition and depending upon the long refractory phase of the ganglion itself, which according to Bishop and Heinbecker (1932) is of the order of 20 to 30 sigma. This assumption is supported by the fact that the phenomenon does not appear during post-ganglionic excitation.

SUMMARY

The fatigability of the pre- and post-ganglionic neurones of the cervical sympathetic was investigated by prolonged stimulation of the pre- and post-ganglionic fibers under the same circumstances. The contraction of the nictitating membrane was used as an indicator.

When the pre-ganglionic fibers were stimulated continuously with an inductorium delivering 42 break induction shocks per second they soon failed to maintain the contraction of the membrane (see figs. 1 and 2).

When post-ganglionic fibers were stimulated under the same conditions, the membrane was maintained in the contracted state (see fig. 2).

That the relaxation observed during pre-ganglionic excitation is not true fatigue is shown by the fact that the membrane contracts again if the frequency of the excitation is decreased (see fig. 4). This pseudo-fatigue is probably related to the Wedensky inhibition; its presence only during pre-ganglionic excitation suggests that it is to be explained by the long refractory period of the superior cervical ganglion.

If pre-ganglionic fibers are stimulated with frequencies adjusted to the ability of the ganglion to transmit impulses, the stimulation may be continuous for at least an hour with no evidence of fatigue. Beyond that period, in our experiments, the observations were complicated by the local injury of the nerve fibers.

In conclusion, I wish to express my gratitude to Prof. Walter B. Cannon for his guidance and help and also for the facilities offered in his department.

BIBLIOGRAPHY

- BISHOP, G. H. AND P. HEINBECKER. 1932. *This Journal*, c, 519.
BRODIE, T. G. AND W. D. HALLIBURTON. 1902. *Journ. Physiol.*, xxviii, 181.
DAVIS, H. AND P. A. DAVIS. 1932. *This Journal*, ci, 399.
EVE, F. C. 1896. *Journ. Physiol.*, xx, 334.
GOTCH, F. 1910. *Journ. Physiol.*, xl, 250.
HOWELL, W. H., S. P. BUDGETT AND E. LEONARD. 1894. *Journ. Physiol.*, xvi, 298.
IOTYKO, J. 1904. *Dictionnaire de Physiologie* par Charles Richet. Felix Alcan. Paris, vol. vi, p. 55.

CAROTID SINUS REFLEXES TO THE RESPIRATORY CENTER¹

I. IDENTIFICATION

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The recent discovery by H. E. Hering and his associates (1) that the carotid sinus is the source of afferent impulses capable of exerting important reflex effects has introduced a new factor into discussions of the regulation of the circulation. It has also led to the discovery that other vegetative functions, including respiration, are profoundly influenced by sinus reflexes. Recent disclosures indicate that the regulation of respiration is accomplished to a remarkable extent through sinus reflexes, and claims are made which, if fully valid, necessitate a fundamental revision in existing conceptions of the nature of respiratory control.

Sinus reflexes to the respiratory center were first investigated by Moissejeff (2) and subsequently by Koch (3) (4), but the most striking evidence and most drastic conclusions have been contributed by Heymans and his co-workers (5) (6) (7) (8).

In briefest résumé, this evidence is as follows: In intact animals (dogs) hyperpnea follows occlusion of the common carotid arteries; it is wholly reflex in origin because the effect is the same even though all branches of the carotids have been previously tied excepting only the lingual arteries, and because section of the sinus nerves completely abolishes the reaction (6). In dogs whose depressor nerves have been cut adrenalin produces apnea only if the sinus nerves are intact, so that adrenalin apnea is purely reflex (6). In experiments in which the sinuses of a dog are perfused with Locke's solution or shed blood, or supplied with blood from a donor animal, rise in endosinusal pressure causes respiratory depression or apnea, fall in pressure hyperpnea, both abolished by section of the sinus nerves (6). Changes in pH or CO₂ content of the Locke's solution perfused through the sinuses of dogs elicit marked respiratory effects; in crossed-circulation

¹ Preliminary reports of these experiments were presented before the Physiological Society of Philadelphia, May 18, 1931 (*Amer. Journ. Med. Sci.*, 1931, clxxxii, 154) and the Federation of American Societies for Experimental Biology, April 28, 1932 (*This Journal*, 1932, ci, 91).

experiments inhalation of CO_2 , nitrogen, or hydrogen by the donor dog leads to reflex hyperpnea in the recipient, and the reflex response may be greater than the direct effect of inhalation of the same gas by the recipient (7). In otherwise intact dogs denervation of the carotids greatly lessens or completely abolishes the respiratory responses to inhalation of CO_2 , nitrogen, or hydrogen (7).

From these experimental results two conclusions of fundamental importance may be drawn: First, the respiratory center (in common with other vegetative centers) would be entirely unaffected by increase in its blood supply, any effect from rise in systemic or cephalic blood pressure being wholly due to reflexes from the sinuses and aorta. Second, the sensitivity of the cells of the respiratory center to changes in their chemical environment must be much less acute than had previously been supposed, the most highly specialized structures in this respect being the afferent end-organs of the carotid sinuses. The first conclusion invalidates Gesell's (9) conception of the regulation of respiration, the second necessitates a drastic revision of Haldane's (10). The respiratory center becomes a relatively insensitive and unimportant synapse in a reflex arc the physiologically specialized part of which is the end-organ in the wall of the internal carotid artery.

The experimental observations of Heymans and his collaborators have been partly confirmed by other workers. The presence of respiratory reflexes aroused by changes in endosinusal pressure is confirmed by Koch and Mark (4) and by Gollwitzer-Meier and Schulte (11) in dogs. The essentially reflex nature of adrenalin apnea is confirmed by Wright (12) in the decerebrate cat. Reflexes aroused by changes in chemical environment of the sinus end-organs, however, could not be demonstrated by Gollwitzer-Meier and Schulte (11) in experiments in which the sinuses of dogs were perfused with Locke's solution or beef blood of varied acidity or gas content. Cromer and Ivy (13) found that aseptic denervation of the sinuses did not appreciably modify the responses of unanesthetized dogs to controlled exercise—an observation which leads them to conclude that any important rôle of sinus reflexes in the regulation of respiration can readily be assumed by other mechanisms.

The experiments now to be described were intended to accomplish, first, a repetition of the observations of Heymans and his collaborators, and second, a subjection of the above conclusions to additional experiments intended further to test their validity. It may be said at once that ample confirmation of the existence and effectiveness of respiratory reflexes aroused by changes in endosinusal pressure has been secured; sensitivity of the sinus mechanism to reduction in oxygen tension of the blood has also been confirmed, but no comparable sensitivity to increase in CO_2 tension could be demonstrated; dependence of the respiratory re-

sponse to anoxemia upon sinus reflexes has also been confirmed, but that to CO_2 excess was found much less dependent upon reflexes (this paper). The results of other experiments, however, do not permit agreement with Heymans' contention that the respiratory (and vasomotor) center is insensitive to increase in its blood supply and that Gesell's conception of respiratory control is invalid (following paper).

I. OCCLUSION AND RELEASE OF THE COMMON CAROTID ARTERIES. Ever since Hering proved that the hypertension which follows bilateral carotid occlusion is due to abolition of inhibitory tone exerted by the sinus reflex mechanism upon the vasomotor and cardio-regulatory centers (1), this procedure has been employed as a simple means for demonstrating the existence and intensity of that influence. Heymans (6) and Koch (4) have used it to demonstrate a similar tonic inhibitory influence upon the respiratory center. Heymans believes that the hyperpnea of carotid occlusion is not due to anemia of the center, as had previously been believed, but is entirely reflex in origin. His evidence is quite convincing: the hyperpnea occurs when all branches of the carotids have been tied excepting the linguals; it is completely abolished by sinus denervation; occlusion of the vertebral arteries has no such effect.

I have repeated these experiments on dogs and cats, using several different narcotics and decerebration in order to obtain an idea of the constancy and intensity of the effects. Eighteen experiments on dogs, with 44 carotid occlusions, and 10 on cats, with 37 occlusions, were deemed acceptable because blood pressure and respiration remained constant and reflexes were active. A number of additional experiments were discarded because of excessively deep narcosis or irregularity of blood pressure or respiration. The narcotics used were barbital, phenobarbital, chloralose, and morphine-urethane. Decerebration was done in 2 dogs and 5 cats by a ligature method (14); the external carotids were tied, but the internal carotids remained open throughout the actual experiment. Blood pressure was recorded from a femoral artery, using 25 per cent sodium thiosulfate or heparin-Ringer as the anticoagulant; a mercury manometer was used routinely. Respiration was recorded quantitatively, in cats and small dogs by the Lieb-Cushny (15) plethysmograph, in larger dogs by a Bohr meter connected to the expiratory side of a valved tracheal cannula with a pneumographic record of the rate. The carotids were occluded by rubber-covered bull-dog clamps, the edges of the wound being kept retracted by weighted hooks; every effort was made to avoid traction upon the vessels or irritation of surrounding tissues when the clamps were manipulated. The occlusions lasted one minute as a rule, preliminary experiments having shown maximal effects to be elicited within that time.

The results are illustrated in figures 1 and 2, which are constructed from the averages of all the observations made excepting those of two recent

experiments on dogs and three on cats. Inclusion of the latter does not materially alter the curves. The individual observations are also shown. Blood pressure was measured every five seconds and the curve is constructed

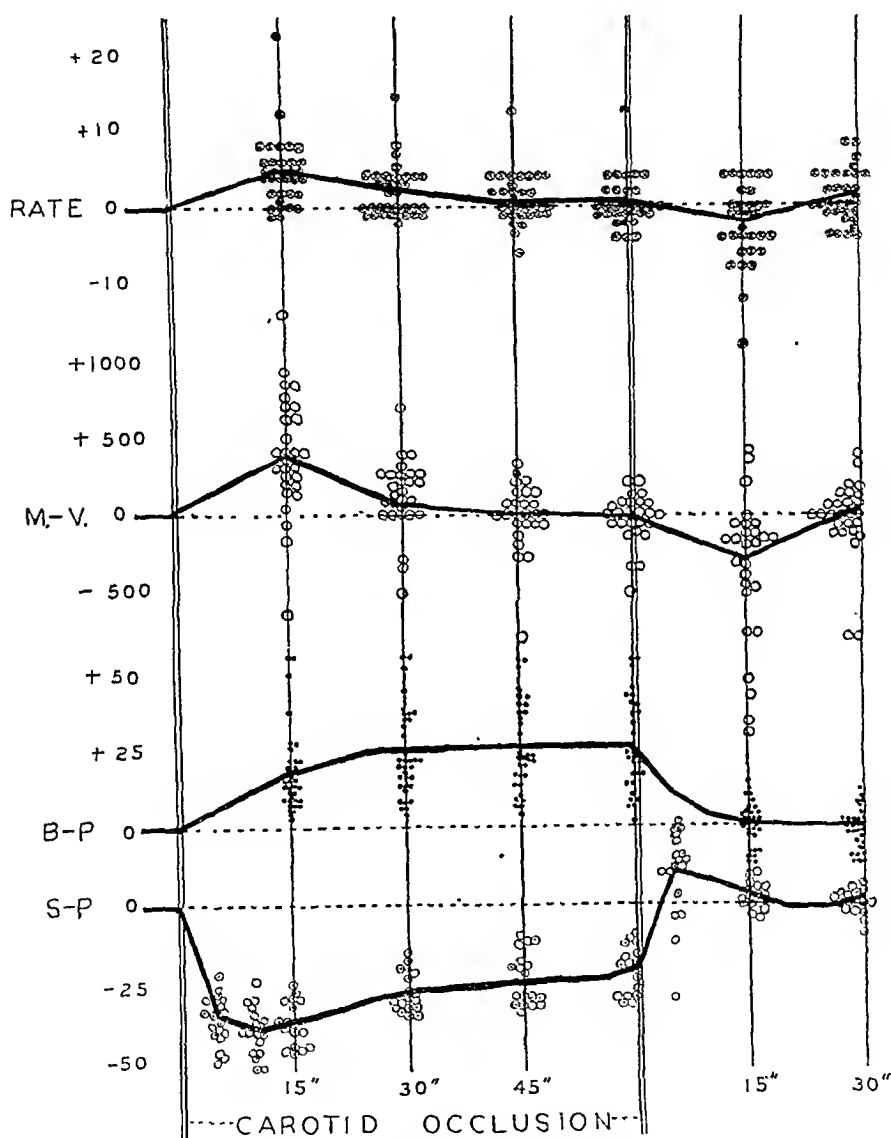


Fig. 1. Effects of carotid occlusion upon circulation and respiration of dogs. Results of 32 occlusions in 16 dogs, of which two were decerebrated; vagodepressor nerves intact in all cases. Curves represent averages of observations (shown as dots or circles) of changes in respiratory rate, *Rate*, respiratory minute-volume, *M-V*, femoral blood pressure, *B-P*, and endosinusal pressure, *S-P*, expressed as changes in respiratory rate or volume (in cubic centimeters) per minute, or in millimeters of pressure, with control value taken as zero.

from those measurements, but only the observations made at fifteen second intervals are shown. Respiration was measured every fifteen seconds.

Two effects were constant, namely, rise in blood pressure during the occlusion, and depression of both blood pressure and respiration upon release of the vessels. Respiratory stimulation upon occlusion was usual but not constant; occasionally respiration was only depressed. There was

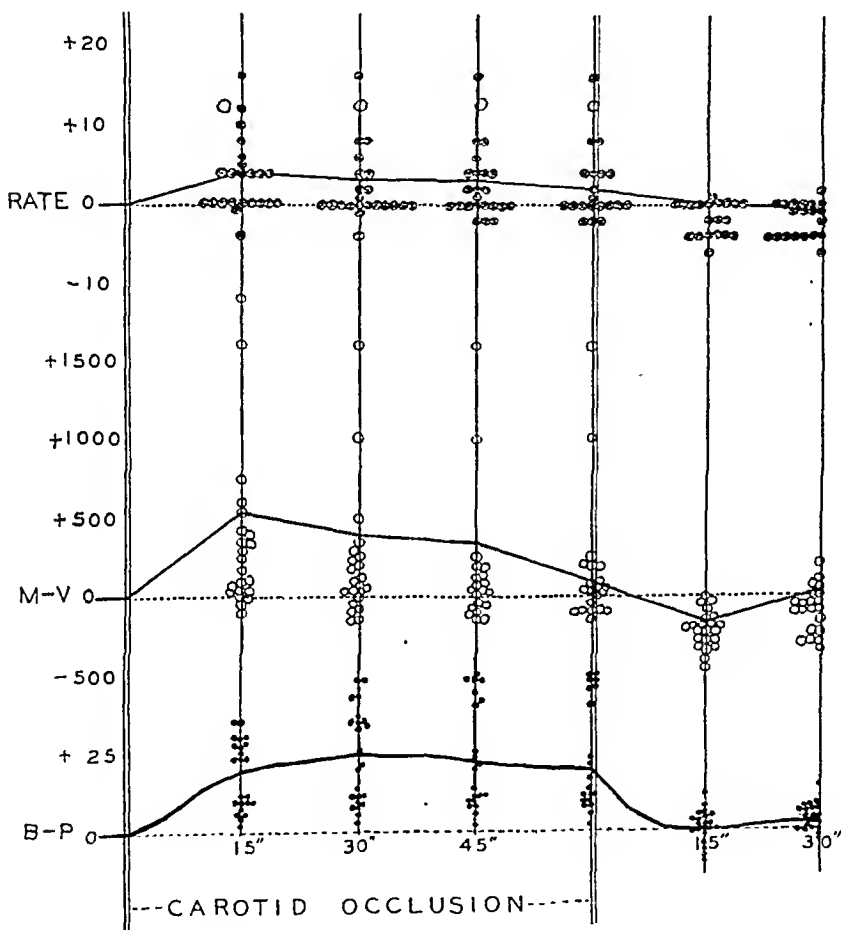


Fig. 2. Effects of carotid occlusion upon circulation and respiration of cats. Results of 33 occlusions in 7 cats, of which 6 were decerebrated; arrangement same as figure 1. Vagodepressor nerves intact.

no constant relation between the intensities of the circulatory and respiratory effects: a marked hypertension sometimes was associated with pure respiratory depression, or a marked hyperpnea with a relatively slight circulatory response. There was nothing to indicate that any of the narcotics used had had an appreciable influence upon the result; the responses were no more marked in decerebrate dogs and cats than in the narcotized animals.

These results are believed to be a fair representation of the regularity and intensity of the respiratory and circulatory effects to be expected from carotid occlusion in the narcotized or decerebrated dog or cat. It is noteworthy that while respiratory stimulation is usual it is not as constant as the circulatory response; more significant is the marked tendency of respiration to return toward normal after the first fifteen seconds of the occlusion, while the hypertension persists as long as the carotids are closed. These differences indicate that if the respiratory and circulatory effects are both wholly reflex in origin, there must be compensatory factors in the former, or else the two effects arise from separate sets of end-organs with different sensitivities to pressure and pulse changes.

Effect upon endosinusal pressure. In the interpretation of the above-described results, information was desired concerning the alterations in endosinusal pressure corresponding with occlusion and release of the carotids. To obtain it a cannula filled with heparin-Ringer solution was tied into the central stump of a lingual artery and connected to a mercury manometer. This was done in four dogs and one cat; in the latter repeated estimations were unreliable by reason of prompt clotting upon occlusion, but the first occlusion yielded good results (fig. 3). The results obtained during 18 occlusions in the four dogs are averaged in the curve labelled *S.P.* in figure 1; the individual observations are also shown. Measurements were made every five seconds, and these are shown for the intervals just after occlusion and release; elsewhere only the observations made at fifteen second intervals are shown, for the sake of simplicity. A typical result is shown in figure 3.

The actual pressures of this average curve were 107 mm. before occlusion, 69 at the lowest point (ten seconds after occlusion), 87 by the end of the minute of occlusion, and 118 five seconds after release.² Expressed as percentages, the abrupt fall in endosinusal pressure amounted to about 36 per cent of the control level of that pressure; the slow rise brought it to about 19 per cent below the control level; the abrupt rise just after release amounted to an increase of 36 per cent above the pressure existing just before release.

Correlating this curve with the others of figure 1 it is evident that the hyperpnea is produced only by a fall of some 35 per cent in endosinusal

² This fall is much less than that found by Heymans (5, p. 541) when he closed the carotid by which a sinus of one dog was joined to a donor animal: his figures show a fall from 150-170 mm. to 60-70 mm. in endosinusal pressure. The difference is probably due to the fact that in Heyman's experiment the outflow from the sinus entered directly into a jugular vein of the donor, so that peripheral resistance must have been abnormally low and collateral contributions to endosinusal pressure were excluded. In a preparation such as his it is impossible to deduce actual endosinusal pressure from the arterial pressure of the donor because of the low resistance on the outflow side.

pressure while the hypertension persists even when the pressure is less than 20 per cent below normal. This suggests that the two reflexes may arise from separate end-organs. An alternative possibility is that the hyperpnea is partly dependent upon anemia of the center, and that the hypertension removes this factor as it also raises endosinusal pressure. In order to decide between these possibilities, sinus denervation was resorted to.

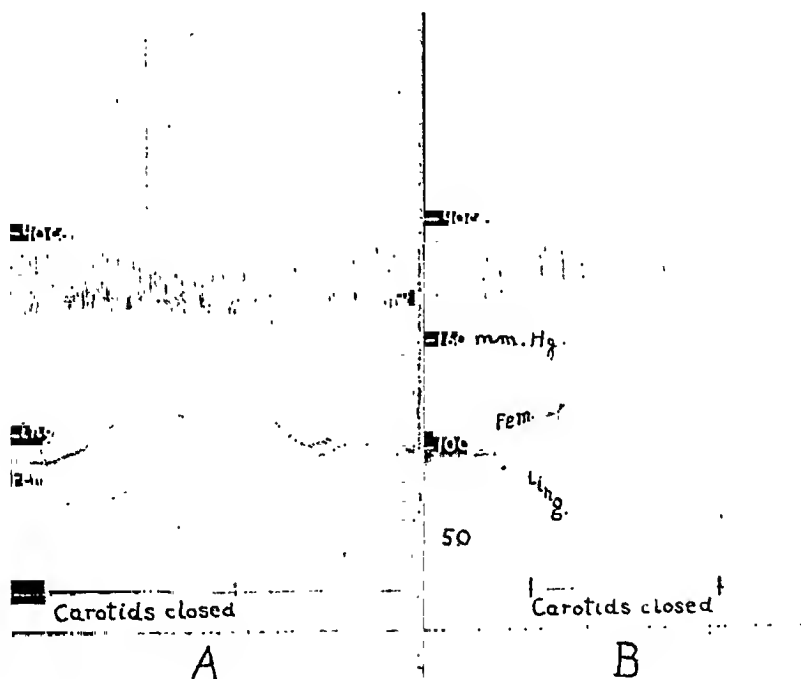


Fig. 3. Effect of carotid occlusion on respiration, blood pressure, and endosinusal pressure.

A. Dog—barbital. Depressor nerves cut (Koch's method), vagi intact. Respiratory plethysmograph. Sinus (lingual) and systemic (femoral) pressures recorded by mercury manometers, zero for both at time tracing.

B. Cat—phenobarbital; vagi and depressors intact. Arrangement same as in A.

Effect of sinus denervation This was done in nearly every one of the experiments but only the results obtained in five dogs and three cats were regarded as valid because in the others there was hypertension or irregular breathing after the denervation. The degree of cerebral anemia produced by carotid occlusion could scarcely be as great in the presence of hypertension as it was before, and irregular breathing indicated a change in the animal's condition that probably was not entirely due to removal of sinus reflexes. The denervation was accomplished in all cases by complete excision, between ligatures, of all attachments of the sinus regions. In each case a series of occlusions was made both before and after the denervation.

The results are well illustrated by the outcome of two representative experiments on dogs, as shown in figure 4. The circulatory response was regularly and (except for a negligible mechanical effect) completely abolished. The respiratory effect was generally reduced and frequently abolished,

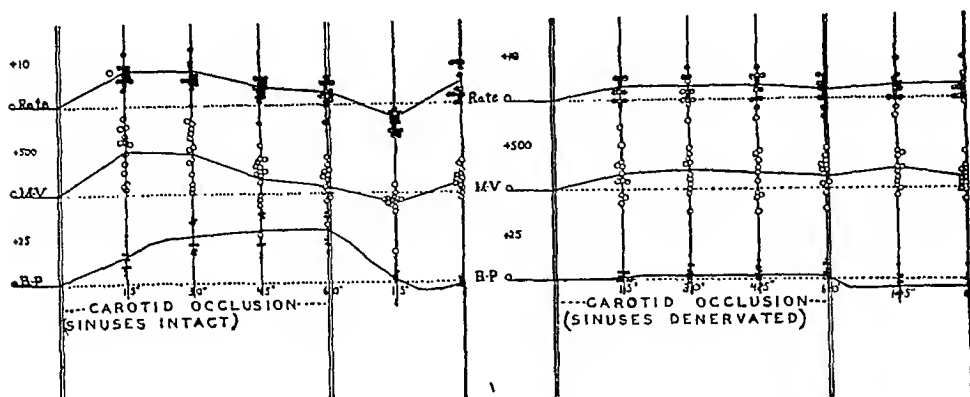


Fig. 4. Influence of sinus denervation upon respiratory and circulatory responses to carotid occlusion. From two experiments on barbitalized dogs. Arrangement same as figure 1.

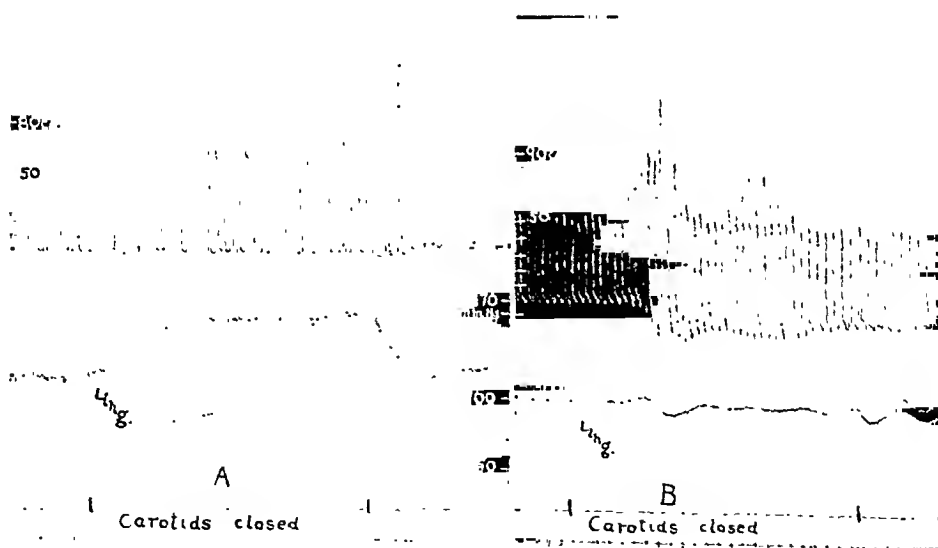


Fig. 5. Influence of sinus denervation on respiratory and circulatory responses to carotid occlusion. Dog—phenobarbital; vagus and depressor nerves intact. Respiratory plethysmograph, femoral and lingual pressures, as in figure 3.

A—sinus nerves intact; occlusion 2 minutes. Respiratory minute-volumes: 2183 cc. before, 2574 cc. during, and 2039 cc. after occlusion.

B—both sinus regions excised; occlusion 2 minutes. Respiratory minute-volumes: 2005 cc. before, 2924 cc. during, 2224 cc. after occlusion.

but in nearly every experiment there was at least one instance of definite hyperpnea from occlusion of the denervated vessels. Exceptionally the effect was even greater after the denervation than before (fig. 5). It must be emphasized that hyperpnea was much more capricious in its occurrence after the denervation, and that instances of its complete absence were much more frequent. This makes the result very difficult to interpret, for if hyperpnea upon occlusion of the denervated vessels is due to central anemia it should be repeatable. On the other hand, if it were due entirely to sinus reflexes it should never occur after the denervation. The only justifiable conclusion is that the hyperpnea of carotid occlusion is largely but not wholly, usually but not always, due to sinus reflexes, and that factors which have not yet been identified operate to make uncertain the results of occlusion of the denervated vessels.

TABLE 1
Carotid and vertebral occlusion—effects upon cerebral blood flow

	REDUCTION IN CEREBRAL VENOUS OUTFLOW ON CLAMPING		
	Carotids	Vertebrals	Both
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Dogs.....	55	33	86
	40		66
	26	13	70
	48	0	
	54	25	83
Average—Dogs.....	45	25	76
Cat.....	55		86

Effect upon cerebral blood flow. Heymans and Bouckaert (6), in support of their contention that the effects of carotid occlusion are wholly reflex in origin, cite experiments in which they find that occlusion of the vertebral arteries has no comparable effects although medullary blood flow should be reduced at least as much by the latter as by the former. I have repeated this experiment on cats and dogs and have confirmed their observation: occlusion of both vertebrals does not cause hypertension or hyperpnea. Their interpretation, however, is not obligatory because they present no evidence that vertebral occlusion actually does reduce central blood flow as much as carotid occlusion does. Before accepting their conclusion it seemed advisable to test by actual experiment the relative effectiveness of carotid and vertebral occlusion in reducing cerebral blood flow.

The method used was that of measurement of venous outflow from the

torcular Herophili, as described in an earlier publication (16). Five dogs were used. The results are summarized in table 1. The percentile reductions represent the average results of three or more occlusions. The results of one earlier experiment on a cat, using the superior caval method (16), are also included. The narcotic was barbital in all cases.

It is not claimed that this method is a precise one, but the results were sufficiently consistent to warrant the conclusion that the degree of cerebral anemia produced by carotid occlusion in the dog is considerably greater than that which follows vertebral occlusion. Absence of hyperpnea from vertebral occlusion may therefore merely mean that the anemia so produced was not intense enough.

SUMMARY

The results of these experiments, while generally confirmatory of Heymans' conclusions, disclose differences between the respiratory and circulatory effects which indicate that the situation may not be as simple as he believed. The evanescence of the hyperpnea, its lack of correspondence with the circulatory effect, and its occasional occurrence after sinus denervation, all indicate that the respiratory effects of carotid occlusion are at least partly or occasionally dependent upon something other than inactivation of the entire sinus reflex mechanism. It is conceded that sinus reflexes are usually responsible for much of the hyperpnea, often for all of it, but the capriciousness of the respiratory effect after sinus denervation points to the intervention of factors which have not yet been identified.

II. RESPIRATORY REFLEXES FROM CONTROLLED CHANGES IN ENDOSINUSAL PRESSURE-PERFUSION EXPERIMENTS. The experiments now to be described represent efforts at repetition of those reported by Heymans and his co-workers (6) (7). Cats and rabbits were used as well as dogs. Both carotids were exposed and, with the aid of a loupe, all vascular branches were tied excepting the external carotids or linguals, in which the efferent cannulae were inserted. The Richards-Drinker (17) pump was used in most experiments; in seven dogs the technic of vessel-to-vessel anastomosis, as employed by Heymans, was utilized.

The procedures used in most of the experiments are shown in diagram in figure 6. This method of crossed-perfusion had the following advantages: first, the blood used for perfusion was taken from and returned to the circulation of a living donor, so that its chemical composition could either be kept constant or altered within limits known to be physiological; second, perfusion pressure being determined by the hydrostatic resistance on the outflow side, it could be set at any desired level or varied within any predetermined limits, depending only upon the position of the mercury reservoir, and regardless of changes in blood pressure in the donor; third, for the same reason the rate and type of pulsation within the sinuses was the

same at all levels of perfusion pressure above zero, so that the influence of changes in pressure could be separated from that of changes in pulsation; fourth, it was possible to test for patent carotid branches in the recipient by the addition of adrenalin to the perfusing blood while maintaining perfusion pressure at a much higher level than systemic: patency even of a tiny vessel was manifested as a rise in systemic pressure. Coagulation of the donor's blood was prevented by intravenous injection of heparin (0.1 gram per kilo). The methods of recording respiration and blood pressure were those described in the preceding section (p. 96).

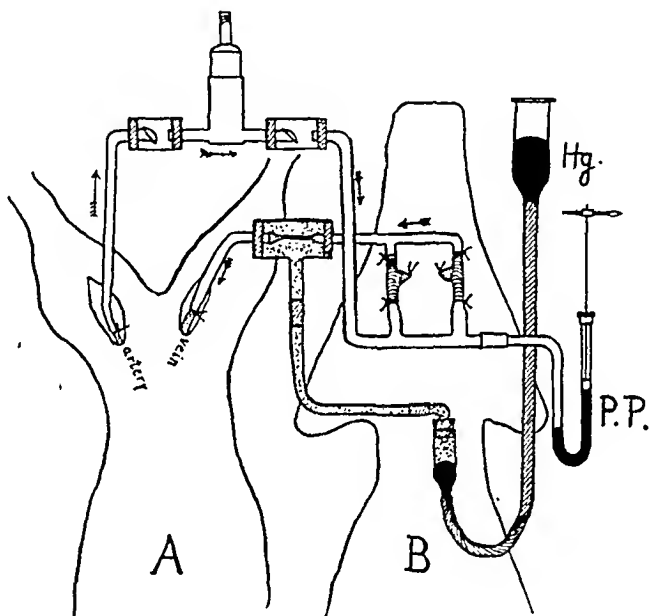


Fig. 6. Crossed-perfusion of carotid sinuses. Blood drawn from a femoral artery of donor (A) is driven by pump through both common carotids of recipient, (B), through cannulae in external carotids or linguals of B, through a thin-walled rubber tube surrounded by saline in a glass chamber, and returned to a femoral vein of donor. External pressure upon rubber section of outflow tube is regulated by adjusting height of mercury leveling-bulb (Hg). Endosinusual pressure is recorded by mercury manometer (P.P.).

The validity of each preparation depended upon reactivity of the sinus mechanism and upon absence of vascular communications between donor and recipient. The first was disclosed by the circulatory effects of alterations in endosinusual pressure, the second by the adrenalin test alluded to above. If the latter indicated a patent vessel, the experiment was not continued until closure had been effected. The additional control of noting the effect of sinus denervation was also used frequently, but only in cases in which it was possible to cut the sinus nerves without mass ligation. Otherwise this is not an adequate check on the dissection because

ligation must of necessity occlude patent vessels, so that the denervation may mean interruption of vascular as well as nervous communication between donor and recipient. The narcotics used were barbital, phenobarbital, amytal, chloralose, and morphine-urethane. A number of experiments were also made upon decerebrate dogs and cats. Of the experiments attempted by this method, 23 on dogs, 11 on cats, and 5 on rabbits were valid in that changes in endosinusal pressure had definite reflex effects and there were no vascular communications between donor and recipient.

The number of experiments was large because in most of them a study was made of "chemical" as well as "pressure" reflexes, and while in the cats it was possible in every case to demonstrate sensitivity to both types of stimuli, it was not possible to obtain "chemical" reflexes in dogs until 18 experiments had been made with this method and 6 by direct anastomosis. Reasons for this will be considered in the following section of this paper.

The results were a complete confirmation of those obtained by Heymans and Bouckaert (6), Koch and Mark (4), and Gollwitzer-Meier and Schulte (11) in that rise in endosinusal pressure caused respiratory depression or apnea with as great uniformity as could be expected in experiments involving as much abnormality as these. Fall in endosinusal pressure likewise caused respiratory stimulation in many cases, but this effect was decidedly less constant than the opposite one of rise in pressure. In further confirmation of Heymans (6), the respiratory (and circulatory) effects of changes in endosinusal pressure were regularly and often very markedly enhanced by section of the vago-depressor or depressor nerves, and were completely abolished by denervation of the sinuses. The results are best illustrated by selected examples from the original records (figs. 7, 8, 9, 10). These are unusually marked effects, but it seems justifiable to regard them as the closest approach to the effects that might be elicited in the absence of deleterious influences such as narcosis, dissection, and artificial circulation. I have also been able to confirm most of Heymans' observations upon dogs with direct vessel-to-vessel anastomosis.

In addition to this confirmation of the results of other investigators, the use of a somewhat different method and of animals that had not previously been used in studies of sinus respiratory reflexes made it possible to secure additional data of some interest. They are as follows:

First, the intensity of the respiratory response to alterations in endosinusal pressure bore no constant relation to that of the circulatory. This is particularly noticeable in comparing the results obtained with cats with those of the rabbit experiments. In the cat the respiratory effects were relatively marked, the circulatory relatively slight, while in the rabbit the circulatory effects were great, the respiratory slight, often completely absent (figs. 9 C and 10). In dogs both types of relation were encountered, but the most striking respiratory effects occurred in animals whose circulatory re-

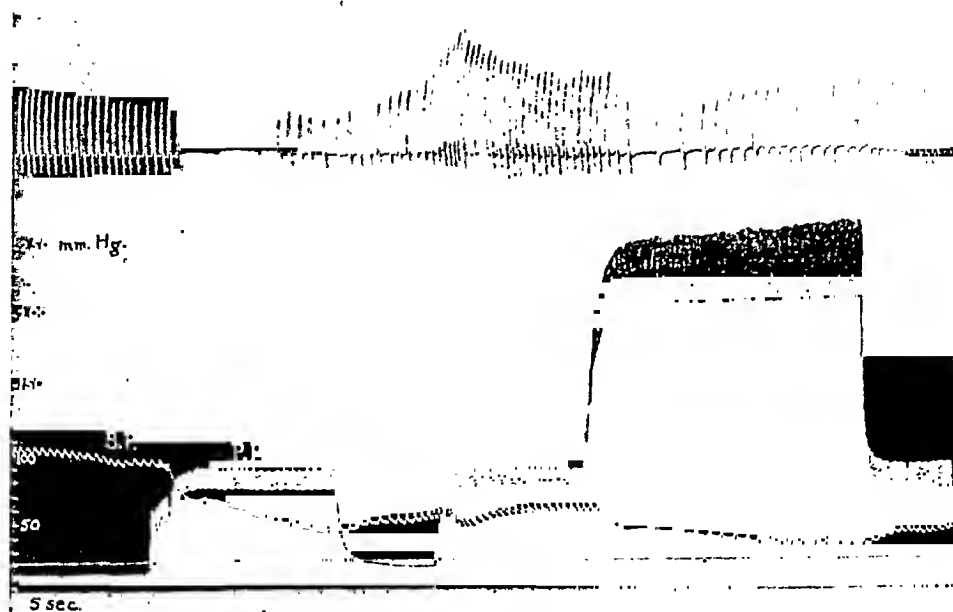


Fig. 7. Effects of alterations in endosinusal pressure—dog. Crossed-perfusion; amytal in donor, barbital in recipient. Vagi of recipient cut, respiration by pneumograph. Perfusion and systemic pressures of recipient by mercury manometers, zero at time tracing.

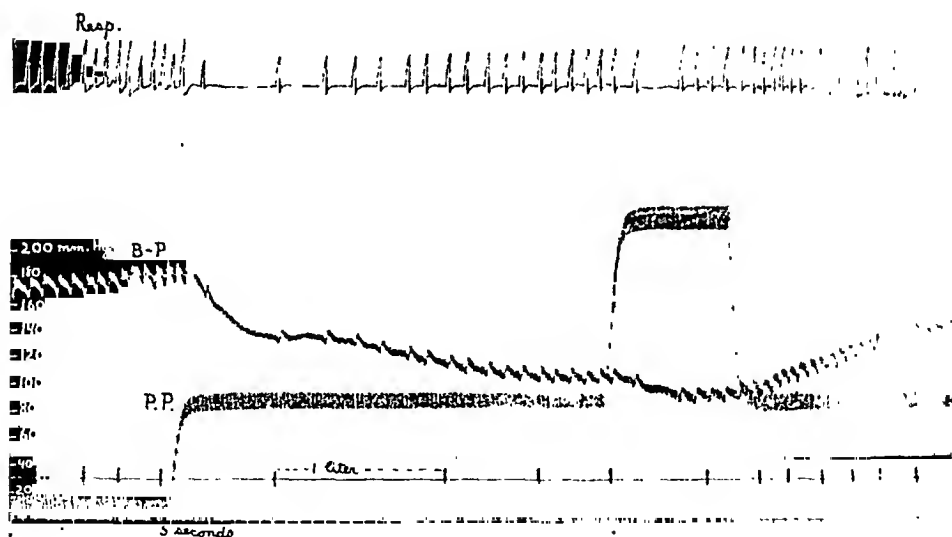


Fig. 8. Crossed perfusion of carotid sinuses—dog. Barbital in donor and recipient. Vagi of recipient cut; mercury manometers, zero at time tracing. Respiration of recipient by pneumograph and by measurement of expired air: each mark of signal (line just above time tracing) represents one liter measured by Bohr meter.

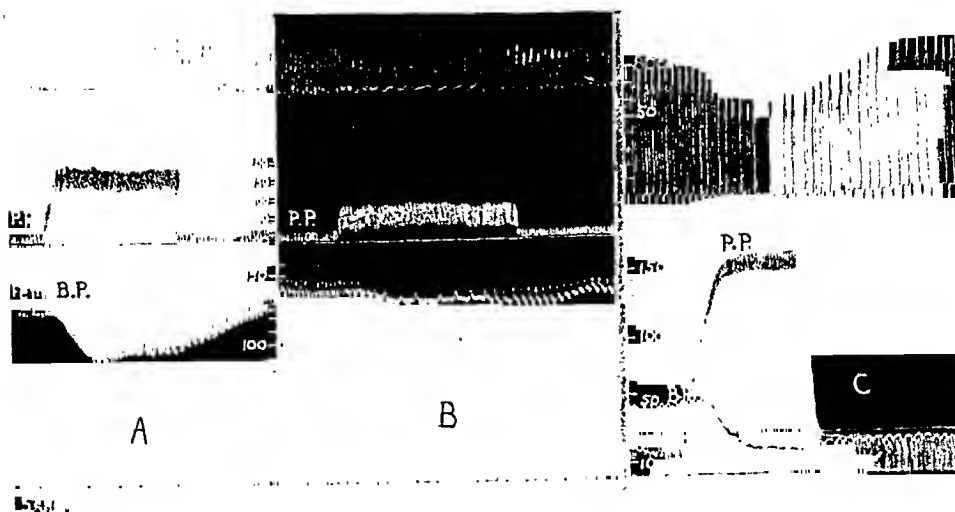


Fig. 9. Perfusion of carotid sinuses—dog and rabbit

A and B from same experiment on barbiturized dog, vagi cut. Sinuses perfused with Locke's solution. Perfusion pressure by membrane manometer, systemic by mercury manometer; respiration by pneumograph.

C—rabbit: barbiturized. Depressor and vagus nerves intact. Sinuses perfused with heparinized rabbit blood. Perfusion and systemic (subclavian) pressures by mercury manometers, zero at time tracing. Respiration by plethysmograph.

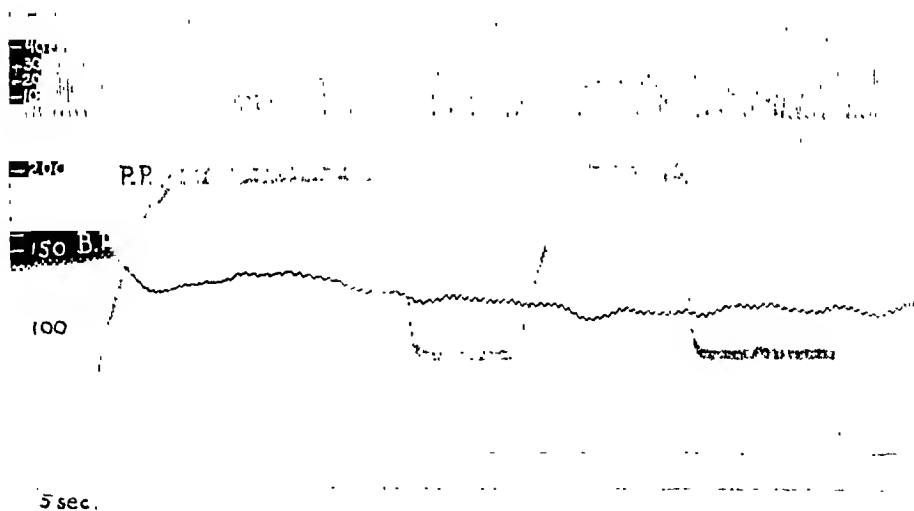


Fig. 10. Crossed-perfusion of carotid sinuses of cat. Barbiturized in donor and recipient; vagi intact. Mercury manometers, zero at time tracing; respiratory plethysmograph.

sponses were relatively slight; in those dogs in which the circulatory effects were most marked the respiratory effects were often comparatively slight.

Second, in all the animals there was evident a striking tendency of respiration to escape from the first effect of alteration in endosinusal pressure when the pressure was maintained at a new level. Blood pressure showed much less tendency to escape, and often showed none at all (figs. 7 and 8).

Third, the respiratory response was essentially a response to change in endosinusal pressure, not to any absolute level of pressure, while the circulatory response was more nearly proportional to the actual pressure level. Thus, a first rise in endosinusal pressure commonly caused apnea even though the actual pressure was lower than arterial, but an additional rise to a high level had less effect, and reduction to the level at which the initial apnea had occurred now caused hyperpnea (figs. 7, 8, and 10). The circulatory effects were more nearly proportional to the actual level of endosinusal pressure.

Fourth, the respiratory effects were greater when the perfusion pressure change began or ended at zero than they were over any other range, even though the actual changes in pressure were much greater in the latter cases. The circulatory effects were more nearly proportional to the actual change in pressure. In decerebrate cats the effect of stopping the pump was commonly to cause intense dyspnea, which, in one case, culminated in violent generalized convulsions; on restarting the pump there was an apnea. These marked effects were elicited by changes in endosinusal pressure amounting to as little as 15 mm. Hg, and were not accompanied by corresponding changes in systemic blood pressure (fig. 11). Similar observations were also made in dogs, though less frequently (fig. 7). It would seem that the respiratory reflexes are more affected by abolition and restoration of pulsation than by actual endosinusal pressure, since the effects were greatest when the pump was stopped and restarted.

Finally, there were instances—most frequent in cats—of distinct respiratory reflexes without any change in blood pressure, and of respiratory stimulation coincident with a marked hypotension following rise in endosinusal pressure (fig. 11).

These observations indicate that the respiratory reflexes aroused by changes in endosinusal pressure do not represent simply the irradiation of excitations intended primarily for the vasomotor center, as Koch (3) believes. It seems most probable that they arise from end-organs that are separate and distinct from those concerned in the circulatory reflexes. Additional reason for this belief is the influence of "chemical reflexes" upon circulation and respiration. These will be considered in the following section of this paper, but the evidence bearing upon the point now under consideration may properly be adduced here. In the decerebrate cat re-

breathing caused intense reflex hyperpnea without any significant change in systemic blood pressure (fig. 13). In the dog anoxemia caused reflex hypotension, while CO_2 excess caused reflex hypertension; both caused reflex hyperpnea (figs. 14 and 15).

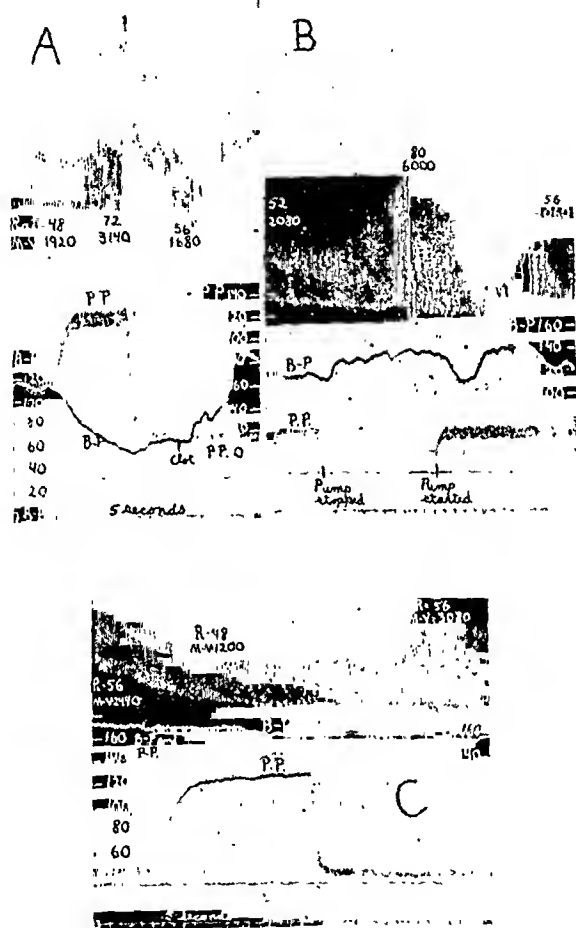


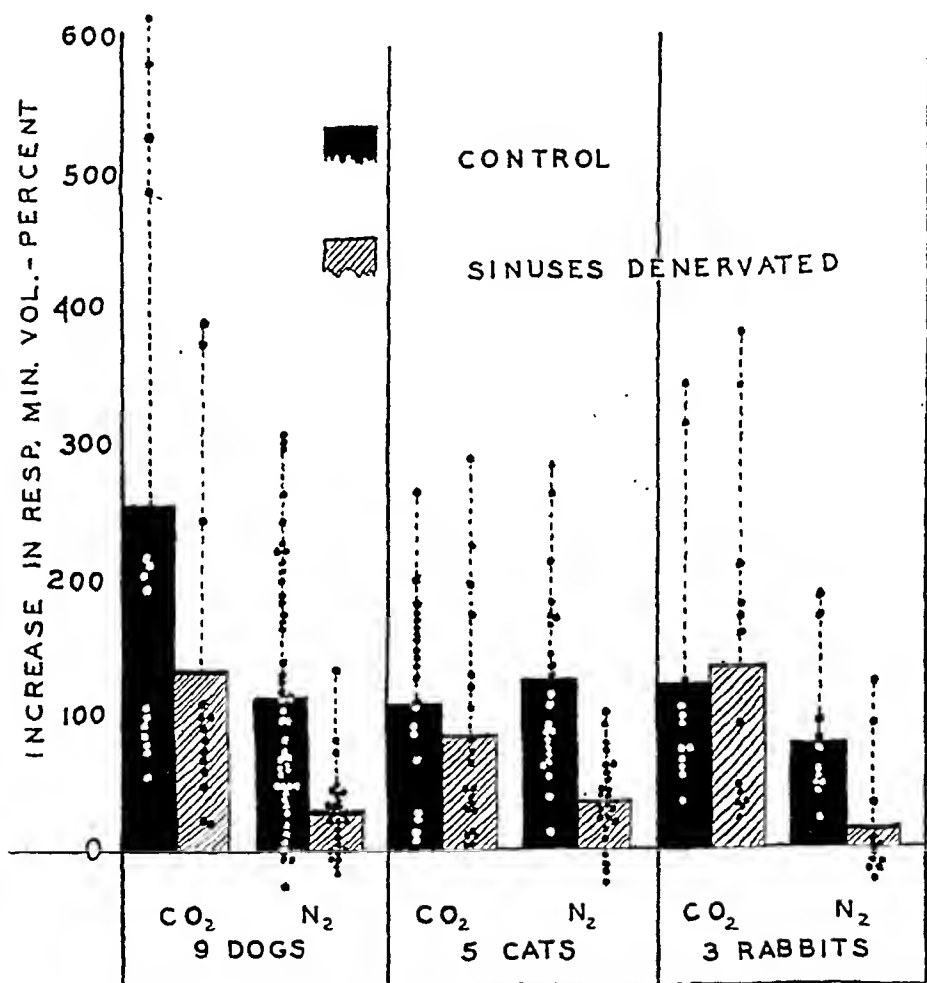
Fig. 11. Crossed-perfusion of carotid sinuses of decerebrate cats

A and B from same experiment: barbital in donor, vagi of recipient intact; mercury manometers, set at different zero levels (calibrations shown); respiratory plethysmograph.

C—another experiment, arrangement same as in A and B; vagi intact.

III. RESPIRATORY REFLEXES FROM CHANGES IN CHEMICAL COMPOSITION OF BLOOD WITHIN THE CAROTID SINUSES. The experiments now to be described represent an attempt at repetition of those of Heymans, Bouckaert, and Dautrebande (7) from which the conclusion was derived that sinus

reflexes play a prominent part in the chemical regulation of respiration. They were of two general sorts: first, determination of the extent to which the respiratory response to inhalation of CO_2 or nitrogen was modified by acute denervation of the sinuses; second, crossed-perfusion and crossed-circulation experiments in which the donor animal was made to breathe



and 3 rabbits. The animals were routinely narcotized with soluble barbital (0.25 gram per kilo intraperitoneally). Registration of blood pressure and of volume of breathing was done as usual (p. 96); the anticoagulant in the arterial cannula was thiosulfate or heparin-Ringer solution. The gases used were pure nitrogen and CO_2 (10 per cent) in oxygen. They were inhaled for one minute as a rule; in some cases the nitrogen inhalation had to be shortened to 20 or 30 seconds because of cardiac effects. A rubber

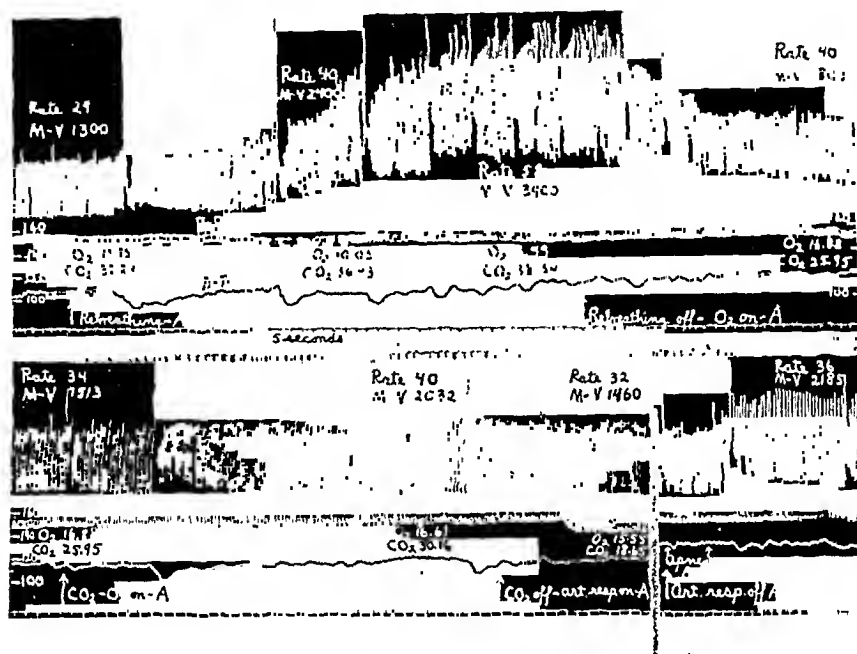


Fig. 13. "Chemical reflexes" from carotid sinuses of decerebrate cat. Crossed perfusion, barbital in donor; vagi intact. Mercury manometers set at same zero level—calibration shown; respiratory plethysmograph. Rate and minute volumes, $M-V$, of respiration of recipient, oxygen and CO_2 content of donor's blood, shown on tracing.

A—rebreathing by donor.

B— CO_2 - O_2 (10 per cent-90 per cent) inhalation by donor.

C—removal of artificial respiration from donor, followed by brief apnea. Follows directly after B.

bag attached directly to the tracheal cannula was used in cats and rabbits, a Douglas bag attached to the inspiratory valve in dogs. In cats and dogs the influence of section of the aortic (vagodepressor) nerves was tested, but in the rabbits only the sinus nerves were cut. After a series of inhalations (usually 5) of each gas, the sinuses were denervated by complete division, between ligatures, of all attachments; in the rabbits the internal carotids were spared because they play a relatively great part in the cere-

bral blood supply of this animal, but in cats and dogs they were cut. After the denervation another series of inhalations of each gas was given.

The results are summarized in figure 12, in which the individual observations as well as their averages are shown in terms of percentile increase

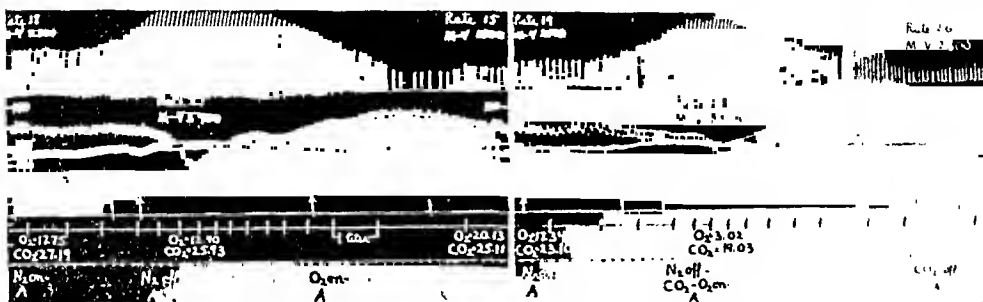


Fig. 14. "Chemical reflexes" from anoxemia in dog. Crossed-perfusion; barbitol in donor and recipient; vagi of recipient cut; mercury manometers set at zero on time tracing; respiration of recipient by pneumograph and measurement of expired air (signal line above time tracing shows each liter expired). Rate and minute-volume of recipient's breathing and oxygen and CO_2 content of donor's blood shown on tracing.

A and B—nitrogen inhalation by donor. In A, oxygen, in B, CO_2 —10 per cent in oxygen, given donor by tracheal catheter. Unfortunately no blood sample was obtained in latter case.

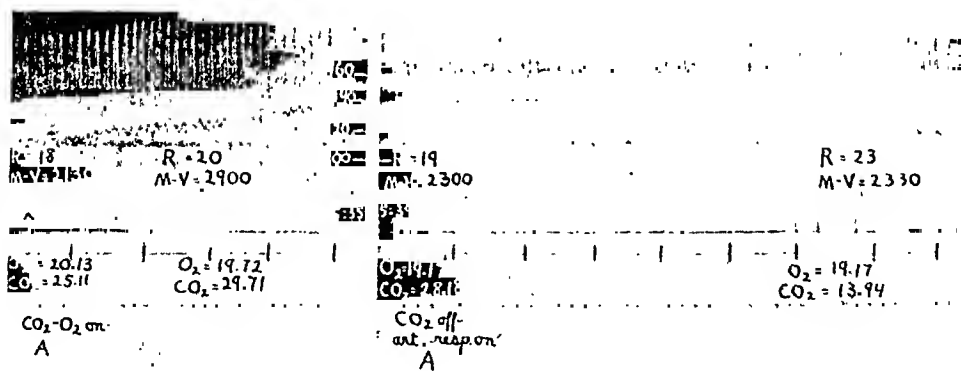


Fig. 15. "Chemical reflexes"—maximal effects of changes in CO_2 content of blood. Same experiment as figure 14. Note relatively slight and brief respiratory effect of CO_2 inhalation (10 per cent in oxygen), with definite hypertension, latter removed by reduction in blood CO_2 content by artificial ventilation; B taken 4 minutes after A, CO_2 inhalation continuing until beginning of B.

in minute-volume of breathing. They clearly confirm the results of the similar experiments of Heymans, Bouckaert, and Dautrebande (7) as far as nitrogen inhalation is concerned: this was almost always made less

stimulant by the denervation, and quite frequently it became purely depressant. The same was true of the circulatory effects also. The response to inhalation of CO_2 in oxygen was not markedly reduced by the denervation in any of the animals, and in the rabbits it was not reduced at all. It may therefore be concluded that in the barbitalized dog, cat, and rabbit the stimulant effects of anoxemia (nitrogen inhalation) are always largely, sometimes wholly dependent upon sinus reflexes, while those of CO_2 excess are mainly central.

The effect of section of the aortic (vagodepressor) nerves in dogs and cats was usually to reduce to the vanishing point the stimulant response to anoxemia remaining after sinus denervation. In cats and dogs there was often some stimulant effect as long as the aortic nerves were intact, but this was not true in rabbits, in which anoxemia was usually purely depressant after sinus denervation alone. From this it appears that aortic reflexes likewise play a considerable part in the anoxic response of the dog and cat, not in that of the rabbit. In no case did aortic and sinus denervation remove the stimulant effect of CO_2 inhalation.

2. "*Chemical reflexes*" in crossed-circulation experiments. These experiments were intended to demonstrate the presence or absence of a physiologically important sensitivity of the sinus end-organs to changes in chemical composition of the blood. The method of crossed-perfusion of the sinuses (fig. 6) was used as well as Heymans' technic of direct anastomosis by means of Payr tubes. The latter was employed in seven successful experiments on dogs; it was tried in cats, but clotting prevented useful results. Crossed-perfusion was preferred because it could be used in cats as well as dogs; because endosinus pressure could be maintained constant in the face of chemical changes in the donor's blood; and because it permitted a conclusive test for the absence of patent carotid branches in the recipient (by means of adrenalin) before the actual experiment began. Respiration and femoral blood pressure of the recipient were recorded as usual. Blood samples, collected from the pump intake, were analyzed for their oxygen and CO_2 content by the manometric method of Van Slyke and Neill (18).

Changes in chemical composition of the carotid blood were induced by subjecting the donor to rebreathing, to artificial respiration, and to inhalation of CO_2 (10 per cent in oxygen), nitrogen, and oxygen. The changes were known to be within physiological limits because they were induced in a living donor. No attempt was made to repeat Heymans' experiments (7) in which saline fluid or shed blood of varied pH or gas content was used for perfusion because the chemical changes used by him were beyond the physiological range. The point of major interest was not the ability of end-organs to respond to a change in their environment, but the physiological significance of such response.

The results were as follows:

In cats results similar to those reported by Heymans et al. (7) were obtained consistently from the start. Attempts at determining the intensity which this effect could attain led to the employment of decerebrate recipients. The most marked effects observed are shown in figure 13. It should be noted that in this animal, in which rebreathing by the donor caused a reflex hyperpnea amounting to a 200 per cent increase in minute volume, changes in CO_2 had only trifling effects; that this difference was not due to loss of reactivity by the animal is shown by the definite reflex hyperpnea caused by the suspension of the donor's breathing upon cessation of artificial respiration (fig. 13) after the CO_2 experiment had been made.

In dogs repeated attempts to obtain similar results were unsuccessful although all of the animals were responsive to changes in endosinusal pressure and some of them were exceedingly so. Heymans (7) (8) having expressed the belief that the "chemical reflexes" probably arise from end-organs separate from those responsible for "pressure reflexes," as suggested by deCastro, the preparation was modified in various ways without success. Finally it was decided to apply to the dog all details of the preparation used in the cat in which positive results had been obtained regularly. The only change consisted in ligation of the occipital arteries at a distance from the parent vessel, using the line of the exposed sinus nerve for reference, whereas in the preceding experiments the occipital artery had been tied close to the carotid for the sake of convenience (fig. 16). As soon as this change was made positive results were obtained consistently in dogs: 5 crossed-perfusions and one direct vessel-to-vessel anastomosis have been made, and the results confirm those of Heymans.

The most striking of these effects are shown in figures 14 and 15. These, as well as the other results with dogs and cats, show anoxemia to be decidedly more effective than CO_2 excess in arousing sinus reflexes. In fact it was possible in this experiment to stop the reflex hyperpnea of anoxemia by substituting 10 per cent CO_2 in oxygen for the nitrogen previously inhaled by the donor (fig. 14).

These results in both dogs and cats conform with those of the denervation experiments in indicating that the sinus mechanism is concerned much more with anoxemia than with CO_2 excess. The effects were proved to be due entirely to reflexes, not only by the routine demonstration of absence of vascular communications between donor and recipient, but also by the effect of section of the sinus nerves without ligation. In the crossed-perfusion experiments endosinusal pressure remained constant, so that the reflex respiratory effects were not contaminated by pressure reflexes.

The outcome of the final experiments on dogs not only furnished confirmation of Heymans' results; it also confirmed his belief that the

mechanism involved in the response to chemical changes is probably distinct from that concerned with pressure changes. Among the 18 experiments in which the occipital arteries of dogs were tied close to the carotids were some of the preparations that were most sensitive to changes in endosinusal pressure (figs. 7 and 9); in none of these were there any definite "chemical reflexes." In the six experiments in which the occipital arteries of dogs were tied at a distance of 5 to 10 mm. from the carotids, there were definite and fully reversible "chemical reflexes" in all, but in only one of them was there outstanding sensitivity to changes in endosinusal pressure; in one there were practically no respiratory responses to changes in pressure, yet there were marked reflex effects from anoxemia in the donor. For these reasons it seemed probable that the receptors involved in "chemical reflexes" are situated in the first portion of the occipital artery in the dog

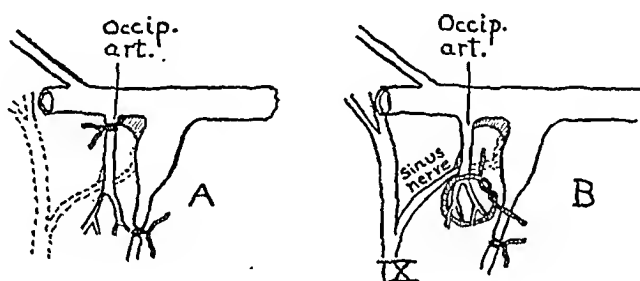


Fig. 16. Change in dissection which furnished positive "chemical reflexes" in dogs.

A—occipital artery tied at origin from carotid: pressure reflexes present, chemical reflexes absent.

B—occipital artery tied beyond line of exposed sinus nerve: pressure and chemical reflexes both present.

and not in the carotid sinus itself. An attempt was made to demonstrate this by a simpler technic, as follows:

Using a vagotomized dog, "pressure reflexes" were elicited by occlusion of the common carotids, "chemical reflexes" by inhalation of nitrogen. A series of occlusions and inhalations was made as a control, then both occipital arteries were tied close to the carotids (fig. 16A) and another series of observations was made. Finally both sinus nerves were cut and a third series of observations was made. Four experiments were performed, barbital being the narcotic.

The results are summarized in figure 17. The chemical response (nitrogen inhalation) was reduced from an average increase of 115 per cent to one of 54 per cent by occipital ligation, while sinus denervation reduced it further to one of 22 per cent: occipital ligation therefore accounted for 66 per cent of the total reduction. The pressure response was changed

by occipital ligation from an average increase of 33 per cent to one of 30 per cent, while sinus denervation reduced it to one of 3 per cent: occipital ligation therefore accounted for only 10 per cent of the total reduction—a change which is not regarded as significant.

These results are presented as evidence that in the dog most of the end-organs which are responsible for the respiratory reflexes aroused by chemical changes in the blood are situated in the first portion of the occipital artery; these reflexes can be greatly weakened or entirely prevented by ligating the vessel at its origin (presumably because this prevents access of the altered blood to the receptors) without interfering appreciably with the respiratory reflex mechanism involved in pressure changes.²

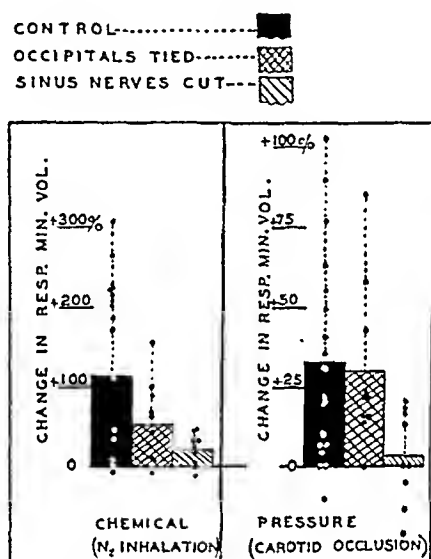


Fig. 17. Influence of occipital ligation upon respiratory responses to chemical and pressure changes—dogs. Blocks represent averages of observations, which are shown as dots. Occipital ligation at level shown in figure 16A.

CONCLUSIONS

1. The respiratory effects of occlusion of the common carotid arteries of dogs and cats, while generally comparable with the circulatory effects,

² This conclusion should be modified in accordance with recent experiments made in Heymans' laboratory (*Annal. de Physiol. et de Physico-Chim. Biol.*, 1931, vii, 207), to which Dr. Heymans has just called my attention. Evidence is there presented that the chemically sensitive receptors are situated in the carotid body which, Dr. Heymans informs me, receives its blood-supply from the occipital artery. My results indicate only that these receptors are supplied with blood from this artery; they do not permit definite localization.

are less constant and less persistent than the circulatory. They are usually but not always purely reflex in origin because sinus denervation does not always abolish them, although it makes them much more capricious in their occurrence. Endosinusal pressure is reduced by about 36 per cent at first, but it rises during the occlusion until it is less than 20 per cent below normal; hyperpnea disappears as endosinusal pressure rises, but hypertension persists, so that both effects can scarcely be due entirely to inactivation of the same sinus reflex mechanism. Carotid occlusion reduces cerebral blood flow by about 45 per cent in dogs, while vertebral occlusion reduces it only by about 25 per cent: the greater effect of the former upon respiration may therefore be partly due to its greater influence upon blood supply of the center.

2. In perfusion experiments upon dogs, cats, and rabbits, rise in endosinusal pressure caused respiratory depression or apnea, while fall in pressure caused hyperpnea; the former effect was more constant and usually more striking than the latter. Section of the depressor nerves enhanced these effects and section of the sinus nerves abolished them.

3. The respiratory effects elicited by changes in endosinusal pressure probably arise from structures that are distinct from those concerned in circulatory reflexes because the intensities of the two effects are often entirely independent; because the respiratory effects are less persistent than the circulatory and less proportional to the actual level of endosinusal pressure than to the level from which the pressure was changed; and because occasionally there may be definite reflex respiratory effects without any circulatory response whatever. The respiratory mechanism appears to be relatively more sensitive to changes in pulsation than to changes in mean pressure, although it is influenced by both.

4. Participation of sinus reflexes in the chemical regulation of breathing is indicated by marked reduction or abolition of the response to anoxemia as a result of sinus denervation and by crossed hyperpnea in a recipient animal when asphyxia or anoxemia is induced in the donor whose blood is used to perfuse the sinuses. In both types of experiment the effects of CO_2 appear to be much more central than reflex.

5. The mechanisms involved in chemical reflexes are probably distinct from those concerned in the responses to pressure changes. In the dog they appear to be located mainly in the first part of the occipital artery.

BIBLIOGRAPHY

- (1) HERING, H. E. Karotissinusreflexe auf Herz und Gefäße. Dresden, 1927.
- (2) MOISSEJEFF, E. Zeitschr. f. d. gesamt. exper. Med., 1926-27, liii, 696.
- (3) KOCH, E. Die reflektorische Selbststeuerung des Kreislaufes. Dresden, 1931.
- (4) KOCH, E. AND R. E. MARK. Zeitschr. f. Kreislaufforsch., 1931, xxiii, 319.
- (5) HEYMANS, C. Rev. Belge des Sci. Med., 1929, i, 507.
- (6) HEYMANS, C. AND J. BOUCKAERT. Journ. Physiol., 1930, lxi, 254.

- (7) HEYMANS, C., J. BOUCKAERT AND L. DAUTREBANDE. *Arch. Internat. de Pharmacodyn. et de Therap.*, 1930, xxxix, 400.
- (8) HEYMANS, C., J. BOUCKAERT AND L. DAUTREBANDE. *Ibid.*, 1931, xl, 54.
- (9) GESELL, R. *This Journal*, 1923, lxvi, 5.
- (10) HALDANE, J. S. *Respiration*. New Haven, 1922.
- (11) GOLLWITZER-MEIER, K. AND H. SCHULTE. *Pflüger's Arch.* 1931, ccxxix, 251.
- (12) WRIGHT, S. *Journ. Physiol.*, 1930, lxix, 493.
- (13) CROMER, S. P. AND A. C. IVY. *Proc. Soc. Exper. Biol. and Med.*, 1930-31, xxviii, 565.
- (14) SCHMIDT, C. F. *Journ. Exp. Med.*, 1923, xxxvii, 43.
- (15) CUSHNY, A. R. *Journ. Pharm. Exper. Therap.*, 1912, iv, 363.
- (16) SCHMIDT, C. F. *This Journal*, 1928, lxxxiv, 202.
- (17) RICHARDS, A. N. AND C. K. DRINKER. *Journ. Pharm. Exper. Therap.*, 1915, vii, 467.
- (18) VAN SLYKE, D. D. AND J. M. NEILL. *Journ. Biol. Chem.*, 1924, lxi, 523.

CAROTID SINUS REFLEXES TO THE RESPIRATORY CENTER¹

II. ATTEMPT AT EVALUATION

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The experiments described in the preceding paper (1) fully confirm the finding of Heymans and Bouckaert (2) that the respiratory center may be profoundly influenced by reflexes aroused in the carotid sinuses by changes in endosinusal pressure. The physiological significance of this reflex mechanism to the control of respiration remains to be determined. According to Heymans (3) (4), the vasomotor and cardio-inhibitory centers are completely unaffected by any physiological changes in flow or pressure in the cephalic circulation; although acute anemia or high external (intracranial) pressure may have direct effects upon them, the physiological responses of these cell-groups to changes in cephalic blood pressure he believes to be entirely due to sinus reflexes. The experiments now to be reported were intended to determine whether this conclusion is applicable to the respiratory center. Direct stimulant effects of acute anemia being conceded, attention was directed mainly to the production of increase in medullary blood flow under such circumstances that sinus (and aortic) reflexes could not contribute to the result. Four different means were employed for the purpose; with two of them a period of acute anemia preceded the increase in blood flow, but with the other two the blood supply of the center was adequate for normal breathing before the flow was increased. The common result of all four sets of experiments was respiratory depression or apnea that could not have been due to known reflexes, that was not always attributable to preliminary exposure to acute anemia, and that is believed to be an indication that the respiratory center is by no means insensitive to alterations in its blood supply, even within physiological limits. In a few other experiments similar observations were made upon the vasomotor center.

1. THE EFFECT OF RELEASE OF OCCLUDED CAROTID AND VERTEBRAL AR-

¹ An exhibit of the results of some of these experiments was given at the meeting of the American Medical Association, June 8-12, 1931 (Journ. Amer. Med. Assoc., 1931, xcvi, 1599).

TERIES. When the common carotid and vertebral arteries are closed simultaneously violent dyspnea results, and if they are reopened while the dyspnea is at its height apnea occurs. These effects have long been known (see 5). The apnea might be due to abrupt increase in blood supply of the brain, or to sudden reinstatement of inhibitory sinus reflexes, or to both influences combined. In order to estimate the importance of sinus reflexes, the carotids were occluded alternately centrally (common carotid) and peripherally (external and internal carotids) to the sinuses, and before and after section of the sinus nerves. The results in four experiments on

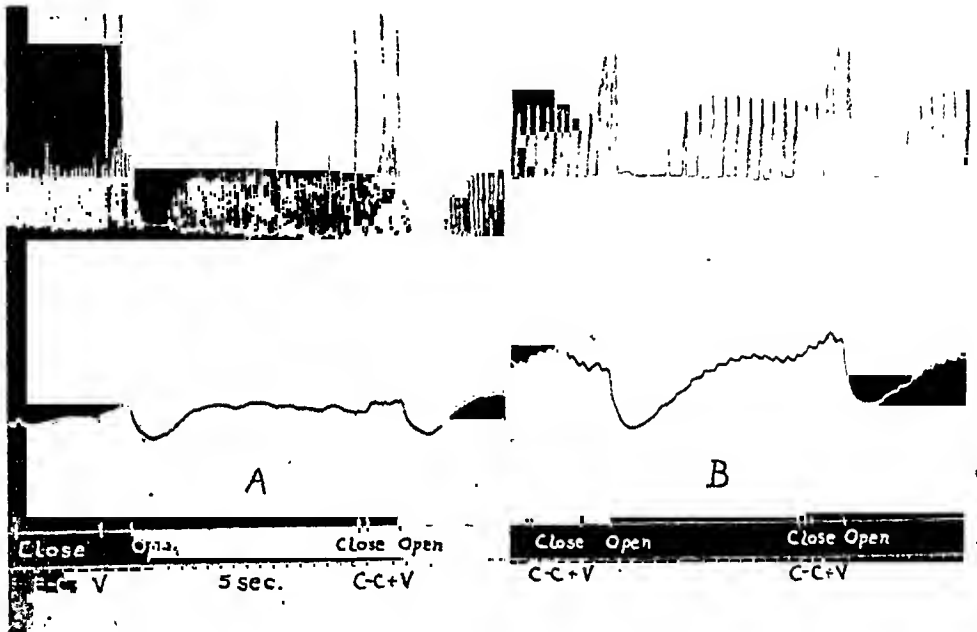


Fig. 1. Apnea upon release of occluded carotid and vertebral arteries—cat. Barbitol; respiratory plethysmograph; femoral blood pressure—zero at time tracing.

A—vagus and sinus nerves intact; internal carotids tied throughout. First occlusion and release—external carotids and vertebrals; second, common carotids and vertebrals.

B—vagi cut, sinus regions excised. Occlusion and release of carotids and vertebrals now produce longer apnea than before.

dogs and three on cats were so uniform and unequivocal that additional observations seemed unnecessary. Characteristic results are illustrated in figures 1 and 2.

In no case was there any sign that carotid sinus reflexes played any part in the apnea produced by release of the occluded vessels. The apnea was just as long when endosinusal pressure rose during occlusion and fell upon release (external and internal carotid occlusion) as it was when the pressure was abruptly raised from a subnormal level (by reopening the

common carotids). Denervation of the sinuses had no influence on the result. To show that the apnea was not due to loss of CO_2 from the blood during the hyperpnea resulting from the occlusion, the experiment was repeated during inhalation of 10 per cent CO_2 . The apnea produced by release of the vessels was not prevented thereby (fig. 2).

This experiment was also tried upon three rabbits narcotized with urethane (two) or ether. In no case was there any hyperpnea upon occlusion or apnea upon release of the carotids and vertebrals, the only effect upon breathing being pure depression by the occlusion and return to normal

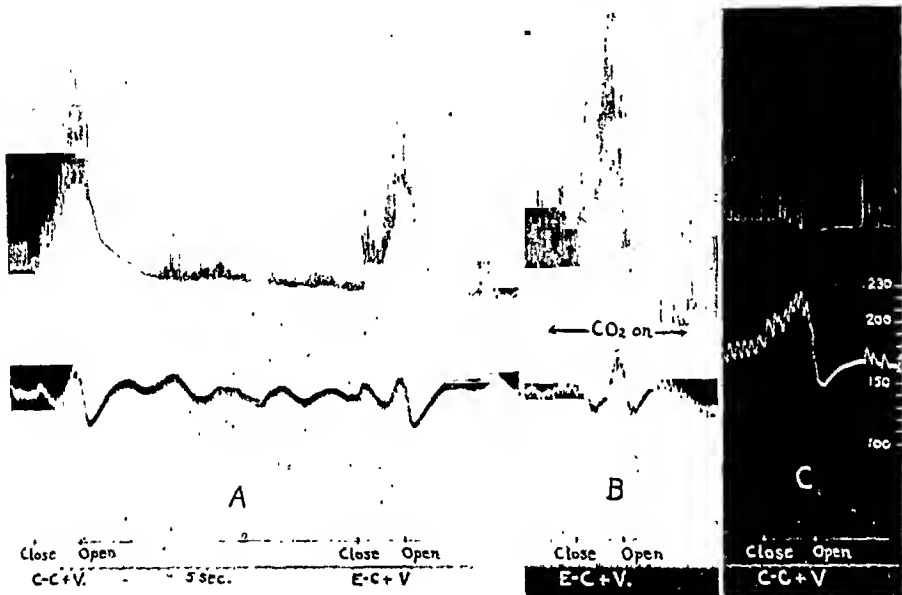


Fig. 2. Apnea upon release of occluded carotid and vertebral arteries—dog. Barbitol; respiratory plethysmograph in A and B, pneumograph in C; otherwise same as figure 1.

A—vagi and sinuses intact, air breathing.

B— $\text{CO}_2\text{-O}_2$ (10 per cent-90 per cent) inhalation throughout—otherwise same as A.

C—vagi cut, sinus regions excised.

upon release. The effects upon blood pressure were of the same nature as those observed in dogs and cats but they were more marked in the rabbits. The reason for this failure of the respiratory center of the rabbit to respond like that of the dog and cat has not been investigated further.

2. THE EFFECT OF SUDDEN REDUCTION IN HIGH CEREBROSPINAL PRESSURE. This procedure was employed as a means for producing a marked increase in cerebral blood flow without the intervention of consensual changes in aortic or carotid pressure. Dogs were used. Cerebrospinal pressure was raised by means of a suspended reservoir of Locke's solution connected

(through a warming coil and a T-tube, to permit instantaneous reduction in pressure) to a metal cannula screwed into a trephined opening in a parietal bone, or to a needle introduced into the cisterna magna. Five experiments were made, with uniform results. Examples are shown in figures 3 and 4.

When cerebrospinal pressure was suddenly raised to a level close to or higher than that of arterial blood pressure, blood pressure rose and respi-

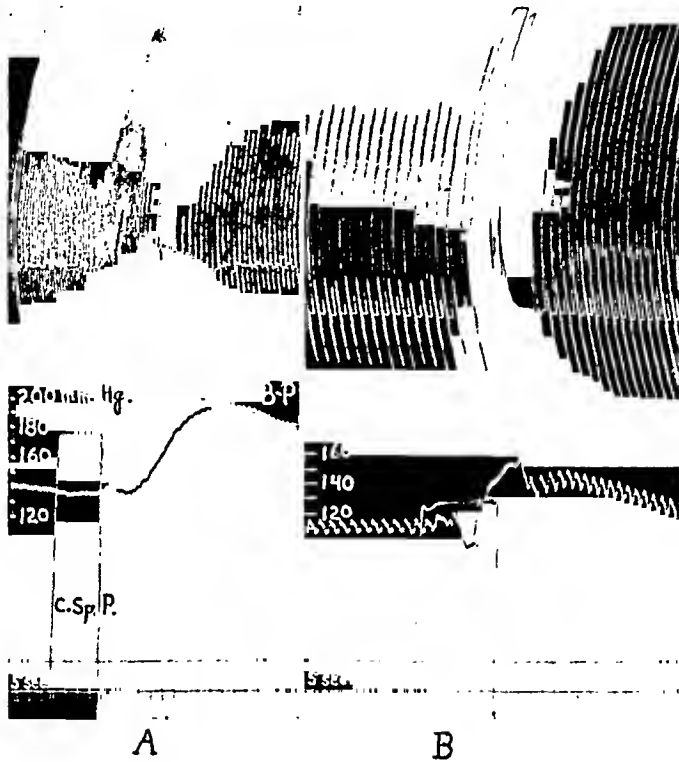


Fig. 3. Apnea upon sudden reduction in high cerebrospinal pressure—dog. Bar-bital; respiratory plethysmograph; cisternal needle connected to mercury manometer to record extramedullary pressure—this and femoral blood pressure both based upon time tracing.

A—aortic nerves cut (Koch's method), both sinus regions excised leaving internal carotids open. Rise and fall of cisternal pressure are shown in rectangular excursion of manometer lever.

B—vagi also cut; note inspiratory spasm without hyperpnea upon elevation, and apnea upon reduction of cerebrospinal pressure.

ration was stimulated. When cerebrospinal pressure was suddenly lowered during this stage there was an immediate apnea. Section of the sinus and vago-depressor nerves did not modify the result. Inhalation of CO_2 throughout the period of elevation and lowering of cerebrospinal pressure diminished but did not prevent the apnea produced by the lowering.

The effects of elevation of cerebrospinal pressure are generally believed to be due to reduction in blood flow through the finer cerebral vessels (6), (16). Sudden reduction of high intracranial pressure may therefore be assumed to act by increasing cerebral blood flow. In any case, neither the hyperpnea of elevated intracranial pressure nor the apnea of sudden reduction of the pressure is dependent upon sinus or aortic reflexes, as shown by the lack of influence from section of the sinus and aortic nerves.

3. EFFECTS OF ADRENALIN AND OF DEPRESSOR DRUGS AFTER SECTION OF SINUS AND AORTIC NERVES. Heymans and Bouckaert (2) were unable to

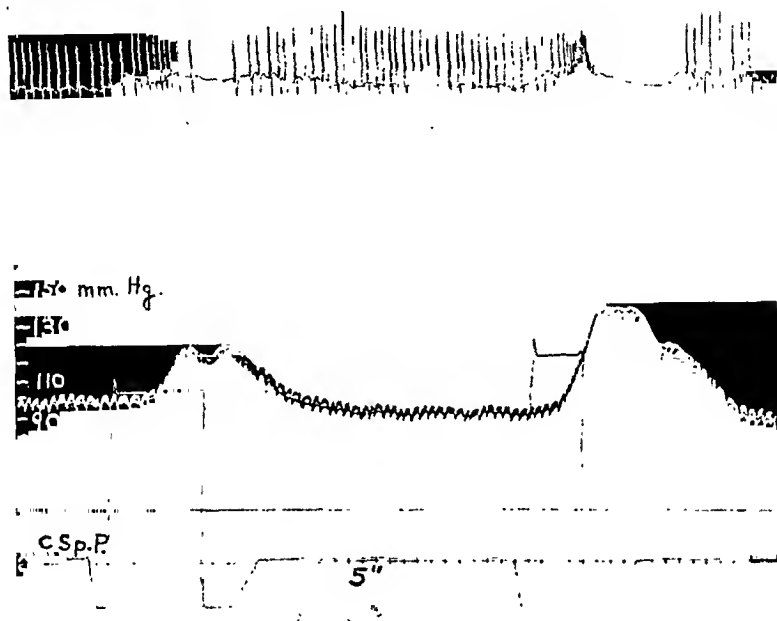


Fig. 4. Apnea upon reduction of high cerebrospinal pressure—dog. Barbitol; pneumograph; cisternal needle; sinus regions excised, leaving internal carotids open; aortic and vagus nerves intact. Otherwise same as figure 3.

produce apnea by intravenous injection of adrenalin in dogs after section of the sinus and aortic nerves. A similar result was obtained by Wright (7) in decerebrate cats. They conclude that adrenalin apnea is wholly due to reflexes from the sinuses and aorta.

This is not supported by the results of these experiments. It is true that in nearly every case in which adrenalin was injected before and after the denervation apnea was no longer produced by it on the latter circumstances. But if the denervation was done at the outset, the first dose of adrenalin commonly caused an apnea. Furthermore, in the crossed-perfusion experiments described in the preceding paper (1) adrenalin was frequently in-

jected intravenously in animals whose carotid systems were isolated and whose vago-depressor nerves were cut. Apnea was a common result, and it was sometimes more marked when carotid pressure was low than when it was high. Nitroglycerine and acetyl choline, injected intravenously in dosage sufficient to lower systemic blood pressure, were also found to be capable in some animals of causing hyperpnea although the sinus and aortic nerves were cut.

Examples of these results are shown in figures 5, 6, and 7. These justify the conclusion that adrenalin apnea is not wholly reflex in origin.

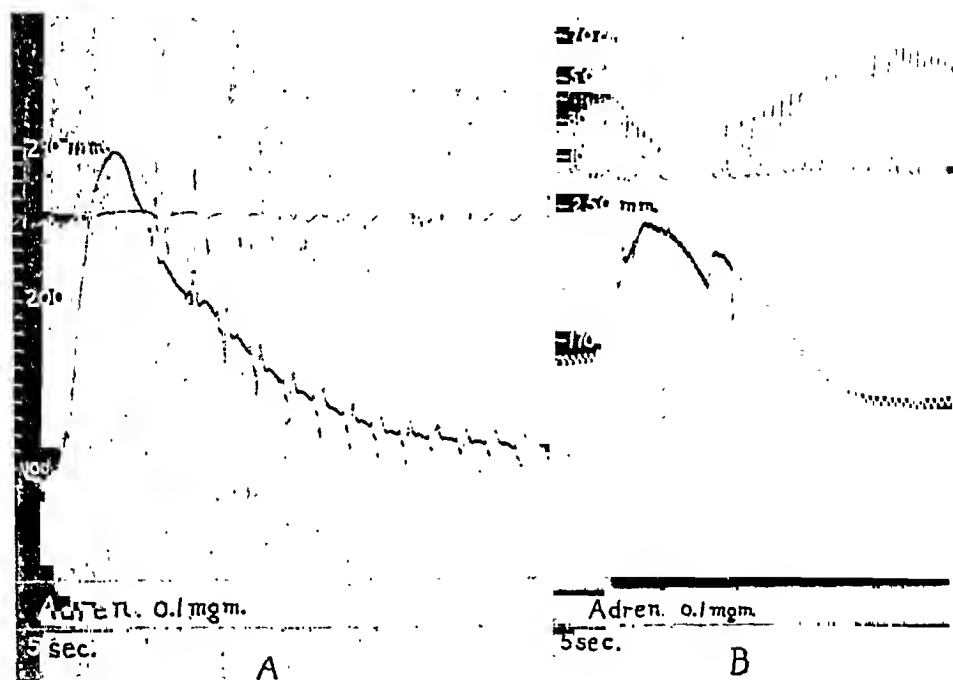


Fig. 5. Adrenalin apnea in decerebrate animals with aortic and sinus nerves cut.
 A—dog; pneumograph; vagi cut; sinus regions completely excised, including internal carotids; external carotids tied; femoral blood pressure.
 B—cat; respiratory plethysmograph; vagi cut; sinus regions excised, including internal carotids; external carotids tied.

The failure of adrenalin to produce apnea after sinus denervation can frequently be duplicated if repeated injections are made with some other operation between them, leaving the sinus nerves intact. The reason for this is not part of the present problem.

Brief mention may also be made of some experiments which indicate that the vasomotor center, like the respiratory, is depressed by adrenalin through mechanisms other than sinus and aortic reflexes. One kidney of a dog was perfused in situ by means of a pump with blood taken from and

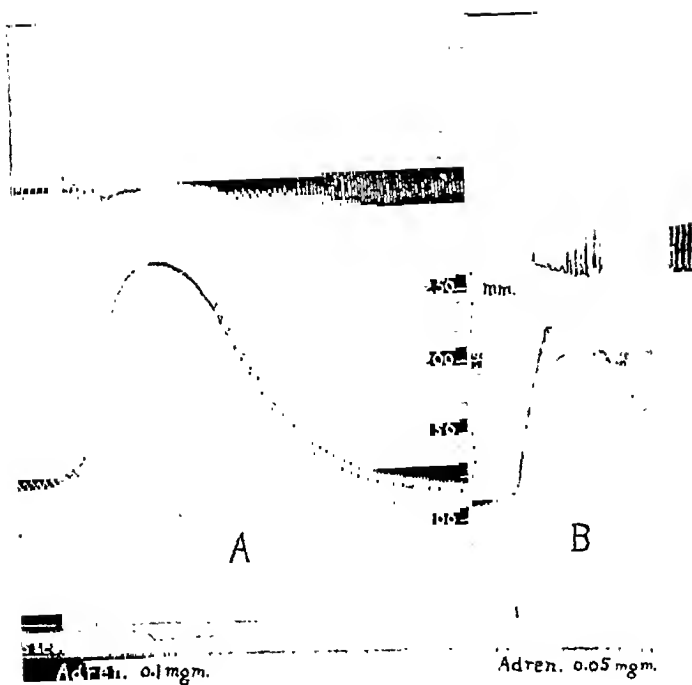


Fig. 6. Adrenalin apnea in animals with aortic nerves cut and sinus pressure under perfusion control.

A—dog—barbital; vagi cut; pneumograph; crossed-perfusion of both sinuses—pump stopped, endosinusal pressure zero.

B—cat—barbital; vagi cut; respiratory plethysmograph; crossed-perfusion of sinuses at constant pressure throughout.

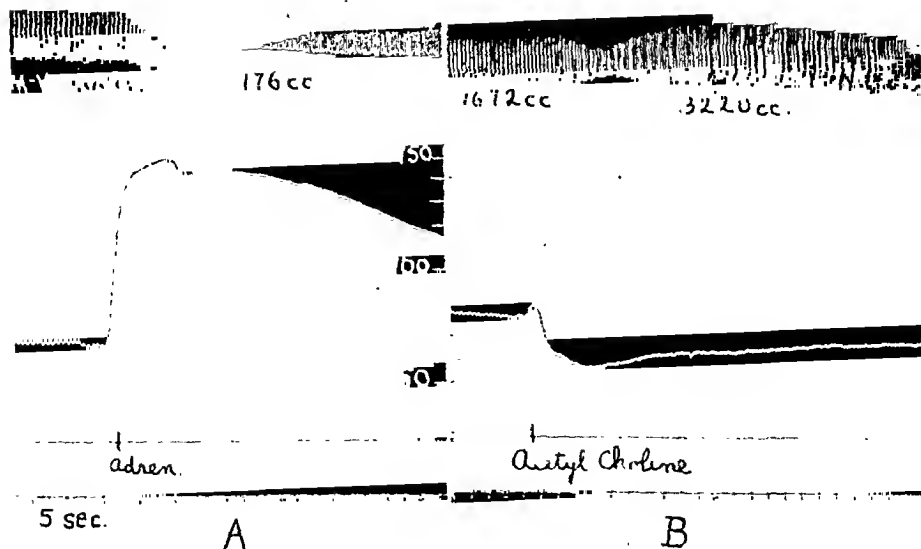


Fig. 7. Respiratory effects of adrenalin and acetyl choline in rabbit with depressor and sinus nerves cut. Barbital; depressors cut, sinus regions excised, leaving internal carotids open; vagi and sympathetics intact; subclavian blood pressure.

A—adrenalin—0.05 mgm. by jugular vein.

B—ten minutes after A; acetyl choline (0.005 mgm. by jugular vein).

returned to the circulation of a donor animal. The vasomotor connections of the kidney were intact, as shown by marked rise in perfusion pressure despite constancy of pump output when asphyxia was induced in the recipient by occlusion of the trachea. Two experiments were made. In both the vago-depressor nerves were cut and carotid sinus reflexes were excluded, in the first by clamping both common carotids, in the second by complete section of all attachments of the sinus region. In one artificial respiration was maintained throughout. Adrenalin, injected intravenously in the experimental animal, caused a fall in perfusion pressure as systemic pressure rose (fig. 8). Since rate of perfusion flow did not diminish, this can only mean decrease in tone of the renal vessels mediated through their

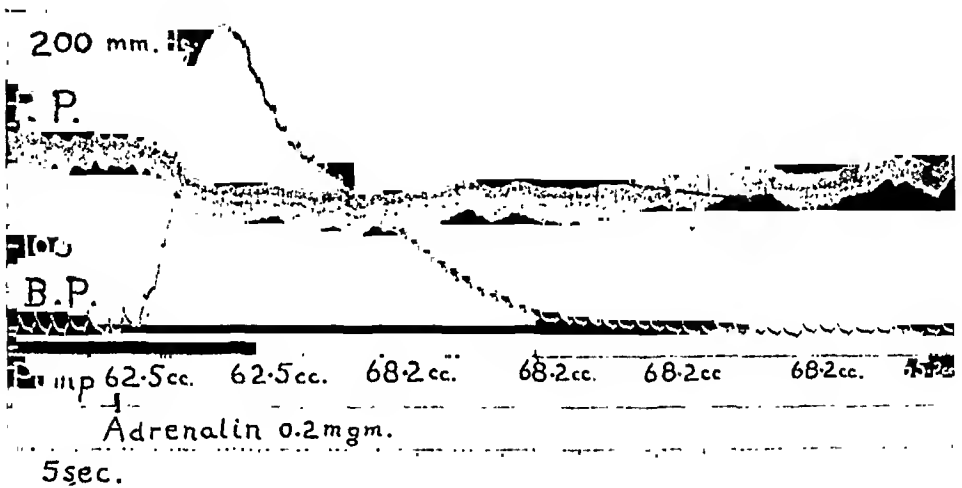


Fig. 8. Depression of vasomotor center by adrenalin hypertension in dog with aortic and sinus nerves cut. Crossed-perfusion of kidney in situ; barbitol in donor and recipient; vagi cut and sinus regions excised in recipient; donor under constant artificial respiration throughout; adrenalin injected into femoral vein of recipient.

Note fall in kidney perfusion pressure (*P.P.*) without reduction in pump output. Respiration of recipient was not depressed by the injection. Pump output (*Pump*) shown in cubic centimeters per minute.

nerve supply, and the indicated vasomotor depression could not have been due to reflexes from the sinuses or aorta.

From the above experiments it is concluded that the respiratory and vasomotor centers are depressed by the rise in blood pressure produced by adrenalin through some agency other than sinus and aortic reflexes.

4. RESPIRATORY EFFECTS OF CONTROLLED CHANGES IN CEREBRAL BLOOD FLOW WITH SINUS AND AORTIC REFLEXES EXCLUDED (PERFUSION OF THE CEREBRAL CIRCULATION). In an earlier publication (5) a report was made of the respiratory responses of cats and dogs to alteration in the rate of flow of blood perfused through the vertebral arteries: respiration, in

favorable experiments, was consistently stimulated by reduction and depressed by increase in vertebral blood flow. Those results are not discredited by the recent disclosures of the importance of sinus reflexes because the common carotids were clamped in every case, and sinus reflexes are presumably inactivated thereby. Aortic reflexes likewise could have played no favorable part in the results because the nature of the experiments demanded a greater withdrawal of blood from the systemic arterial circulation when vertebral flow was increased, so that aortic pressure was necessarily decreased whenever vertebral flow was increased. The instances

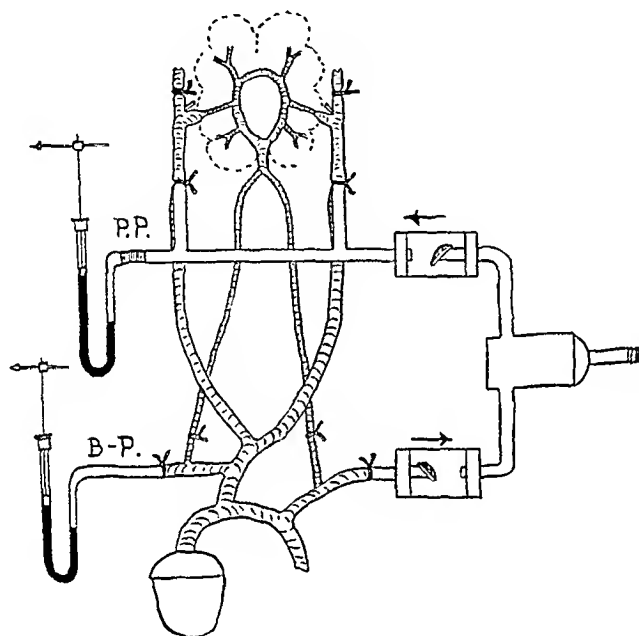


Fig. 9. Method for perfusion of brain of rabbit with animal's own blood. Outflow of pump enters cannulae tied into common carotids; external carotids and vertebral are tied; sinus regions are excised, leaving internal carotids open. Pressures recorded by mercury manometers.

cited in the paper referred to (5, fig. 9) are therefore valid evidence that the respiratory center is directly influenced by changes in its blood supply in either direction.

It seemed advisable, however, to make absolutely certain that aortic and sinus reflexes could be excluded without abolishing the respiratory responses to changes in cerebral perfusion flow. For this purpose a series of experiments was made in which the brain was perfused by way of the internal carotids, before and after section of the sinus nerves, and with the aortic nerves divided. Rabbits were used because their internal carotids are relatively large, because their intracranial circulations are not in free

communication with extracranial areas, and because section of the aortic nerves is readily accomplished without division of the vagus trunks.

The method is shown in diagram in figure 9. The narcotic was soluble barbitol (0.25 gram per kilo intraperitoneally). The carotid and subclavian arterial systems were dissected free, all branches of the latter being tied excepting the vertebrals which were prepared for subsequent occlusion by means of loose ligatures. The external carotids and all carotid branches other than the internal carotids were tied. Blood was taken from one subclavian artery to supply the Richards-Drinker pump, by which it was driven through the cephalic ends of the common carotids. A by-pass to a jugular vein was provided to prevent perfusion of the brain with saline or with stagnant blood at the outset. Clotting was prevented by intra-

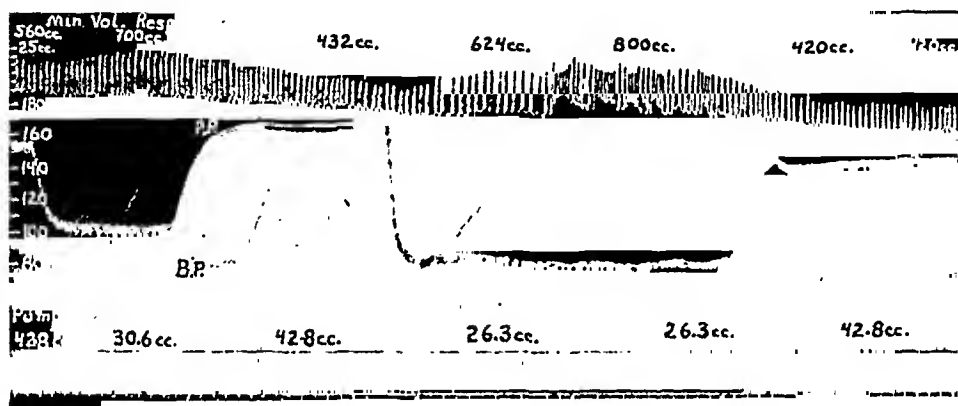


Fig. 10. Respiratory effects of alterations in internal carotid perfusion flow in rabbit. Barbitol; respiratory plethysmograph; depressor and sinus nerves cut; perfusion pressure, "P.P." and subclavian blood pressure, "B.P." based upon time tracing. Respiratory minute-volumes and cerebral blood flow, *pump* are shown as cubic centimeters per minute.

venous injection of heparin (0.1 gram per kilo). Respiration was recorded plethysmographically and the aortic nerves were cut at the start of each experiment.

The results of the four successful experiments of this nature are summarized in table 1, and some of the most striking effects are illustrated in figure 10. In the first of these experiments the animal's circulation failed after the sinuses were denervated, but the results up to that time are included because they illustrate the sort of effect to be expected when sinus reflexes are operative. In the last one the sinuses were denervated at the start, while in the other two definite results were obtained both before and after the denervation. Table 1 also shows a summary of the results of the most favorable of the earlier vertebral perfusion experiments.

These results are presented as the final piece of evidence that the respiratory center is directly affected by changes in its blood supply.

TABLE 1

Respiratory effects of changes in cerebral perfusion flow

Barbital narcosis in all cases. Figures for cerebral blood-flow and respiratory minute volume (M-V) represent cubic centimeters per minute; respiratory rate per minute also shown (Rate). Depressor nerves cut in rabbits, intact in dogs and cats.

	PERFUSION VIA	INCREASE IN CEREBRAL BLOOD-FLOW										DECREASE IN CEREBRAL BLOOD-FLOW										SINUS NERVES
		Effect on respiration					Per cent change in					Effect on respiration					Per cent change in					
		From		To		Rate	M-V	Rate	M-V	Flow	Resp. M-V	From		To		Rate	M-V	Rate	M-V	Flow	Resp. M-V	
		From	To	From	To							From	To	From	To							
Rabbits	Int. carotids	16.3	28	72	432	56	168	+72	-61	22.9	16.3	72	432	72	432	72	720	-29	+66	Intact		
		16.6	48.4	68	340	60	120	+191	-65	48.4	16.4	60	120	60	480	480	-195	+300	Intact			
		3.6	20.4	52	312	76	228	+466	-26	11.2	3.6	76	304	52	310	310	-211	+2	Cut			
		17	29.7	64	2,560	64	2,240	+70	-11	23.1	17	72	2,520	64	2,560	2,560	-26	+2	Intact			
		25.6	39	40	1,280	32	640	+52	-50	42.8	26.3	24	432	40	800	800	-38	+85	Cut			
		18.7	22.4	40	1,080	32	800	+20	-26	34.9	24.8	60	1,800	60	2,400	2,400	-29	+33	Cut			
Cats	Vertebals	14.5	22.4	52	1,352	48	1,056	+54	-21	35	20	52	1,560	56	1,940	1,940	-43	+24	Inactive (carotids closed)			
		24.2	47.6	80	1,920	80	800	+97	-58	47.6	19.5	140	1,960	140	2,380	2,380	-59	+21	Inactive (carotids closed)			
		40.5	57.7	92	1,840	100	900	+42	-40	57.7	33.7	100	900	76	1,900	1,900	-42	+111	Inactive (carotids closed)			
		30	55.5	44	1,320	40	800	+85	-40	60	41.6	48	1,200	56	1,680	1,680	-31	+40	Inactive (carotids closed)			
		33.3	38.4	128	5,120	140	2,800	+15	-45	38.6	23.8	140	2,800	128	7,040	7,040	-38	+150	Inactive (carotids closed)			
Dogs	Vertebals	47	50	48	1,680	44	1,320	+6	-21	50	40.5	48	1,440	48	1,584	1,584	-19	+10	Inactive (carotids closed)			
		50	56.6	40	2,080	40	1,840	+13	-11	41.6	23.1	32	1,760	40	2,600	2,600	-44	+48	Inactive (carotids closed)			
		21.2	23	64	3,200	44	2,420	+8	-24	57.7	35.7	40	1,600	52	2,184	2,184	-38	+37	Inactive (carotids closed)			

5. INTERPRETATION OF RESULTS. From the experiments reported in the preceding sections of this paper one conclusion can be drawn with certainty. It is that sinus and aortic reflexes are not the only agency concerned in the respiratory effects of changes in cephalic or systemic blood pressure. The further conclusion that the respiratory center is directly affected by alterations in its blood supply, in the manner stipulated by the hypothesis of Gesell (8), I believe to be justified, for the following reasons: First, the fact that adrenalin apnea occurs in decerebrate animals with sinus and aortic nerves cut localizes the responsible factor or factors in the brainstem and excludes reflexes from the external carotid area, the possible importance of which has been mentioned by Koch (9). Second, the four sets of experiments just reported had only one evident factor in common, that being alteration in blood supply of the center; since the common result was respiratory depression with increase and respiratory stimulation with decrease in flow it is most probable that the respiratory effect was the result of the change in flow. This can be proved only by exclusion of all other possibilities as they arise; all that are known to me have been excluded.

Even though it be true that alterations in cerebral blood flow have direct effects upon the respiratory center, there can be no doubt concerning the ability of reflexes aroused in the sinuses by changes in endosinusal pressure to produce the same sort of respiratory effect. The observations of Moissejeff (10) and Heymans and Bouckaert (2) have been so regularly confirmed by subsequent workers (11) (12) (1) as to prove this point beyond question. It would appear therefore that the respiratory effects of changes in cephalic blood pressure might be due either to sinus reflexes, or to changes in blood supply of the respiratory center, or to both influences combined. If the conclusions which Heymans (4) has drawn with respect to the normal regulation of the vasomotor and cardio-inhibitory centers are applicable to the regulation of the respiratory center, the direct effects of alteration in blood supply must be relegated to the background and regarded as purely pathological, all respiratory effects of changes in cephalic blood pressure under physiological conditions being attributed entirely to sinus reflexes. This would mean that the sensitivity of the respiratory end-organs of the sinuses to changes in endovascular pressure is vastly greater than that of the cells of the center to the changes in their internal environment produced by changes in their blood supply.

This is a question of considerable theoretical importance because it involves the fundamentals of respiratory control. If the reflex factor is all-important and responsivity to changes in blood flow is purely pathological, it follows either that the cells of the center have such a low metabolic rate that only extreme changes in their blood supply have an appreciable influence upon intracellular conditions, or else the cells are very much less

sensitive to changes in their internal environment than has hitherto been supposed. There is a considerable amount of evidence (see 13, 8, 14) against both of these deductions, and even though this evidence is indirect and inconclusive, it can scarcely be abandoned until there is convincing evidence of the necessity therefor. I do not believe that any evidence has as yet been adduced which compels the belief that these reflex influences are at all essential to the regulation of breathing, however important they may be to the regulation of blood pressure; to this there is only one exception.

The exception is the hyperpnea of anoxemia, which appears to be mainly if not entirely reflex in origin (15) (1). The hyperpnea of excess CO_2 , on the contrary, is much more central than reflex in origin (15) (1), and the same appears to be true of the respiratory responses to changes in pH of the blood: Heymans and co-workers (15) found that reflexes were not aroused over the range pH 7.1 to 7.6, within which any physiological changes would fall. Consequently the only change which these recent disclosures create in the Haldane conception of respiratory control is an explanation of the differences between anoxemia and CO_2 -excess as respiratory stimulants: the former is mainly reflex in action, the latter mainly central. The fundamental feature of Haldane's conception, namely, the exquisite sensitivity of the center to changes in CO_2 tension in the blood (13), is not affected because the action of CO_2 remains central. Furthermore, since respiration responds to extremely slight increases in blood CO_2 tension while relatively enormous reductions in oxygen tension are required to produce a reflex hypernea, it follows that the cells of the center are vastly more sensitive to their most effective stimulus (CO_2) than the sinus chemical organs are to theirs (oxygen-lack). The central organ therefore remains the most highly specialized part of the respiratory mechanism, so far as chemical regulation is concerned.

Apart from this, sinus reflexes to the respiratory center have not been shown to be necessary to any feature in respiratory control. While their effectiveness cannot be denied, they do nothing that cannot be accomplished quite as well without them by some other mechanism. Thus, the production of apnea by adrenalin in animals in which sinus and aortic reflexes were excluded (figs. 5, 6, 7) shows that increase in central blood flow is fully capable of accomplishing the same end-result as the reflexes. The experiments in which the cerebral circulation was under perfusion control (table 1) showed instances of respiratory depression from increases in cerebral blood flow amounting to as little as 6, 8 and 13 per cent of the preëxisting flow. The smallest rises in endosinusal pressure by which respiration was reflexly depressed in my experiments were 12, 16 and 23 per cent of the existing arterial blood pressure. While there is no common ground upon which these two sets of figures can be compared, they indicate

at least that the respiratory center may be quite as sensitive to increase in its blood supply as the respiratory reflex mechanism is to increase in endosinusal pressure. From the standpoint of relative power of the two influences, there is no doubt that the excitant effect of acute anemia can overcome the strongest possible reflex inhibition (fig. 11); also, the respiratory depressant action of increase in central blood supply may be decidedly greater than the reflex inhibition produced by elevation of endosinusal pressure to a comparable level (fig. 12).

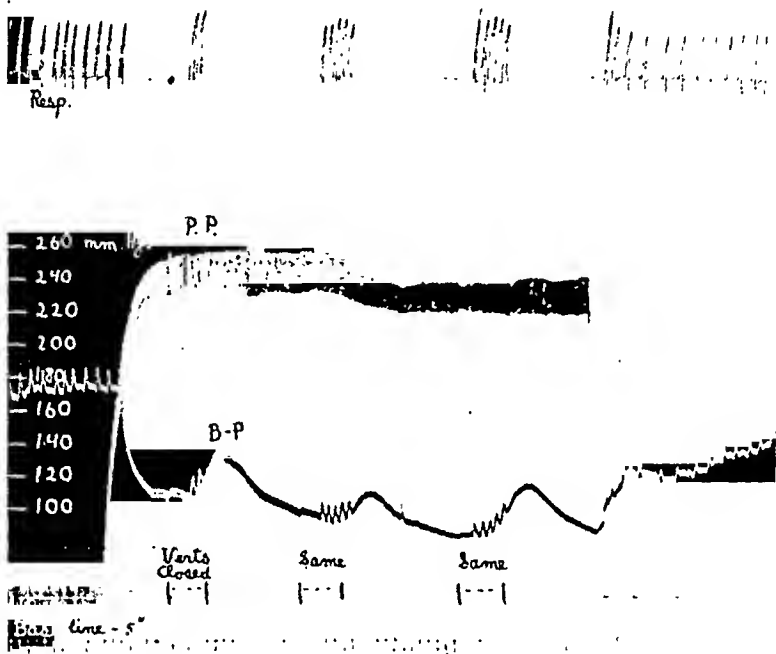


Fig. 11. Acute cerebral anemia overcoming inhibitory sinus reflexes—dog. Barbitol; vagi cut; pneumograph; sinuses perfused with defibrinated dog blood; current of oxygen by tracheal catheter throughout—in absence of vertebral occlusion there were no respiratory efforts when endosinusal pressure was kept elevated. Note that each time vertebral arteries were closed respiration and circulation were promptly stimulated.

Drawing thus upon selected examples it is possible to show that Heymans' conclusion—namely, that vegetative centers are entirely unaffected by changes in their blood supply within physiological limits (4)—is inapplicable to the respiratory center. But when the available evidence is considered as a whole, it becomes evident that the direct sensitivity of the respiratory center to changes in cephalic pressure is more variable than that of the sinus reflex mechanism. This is illustrated by the greater difficulty in eliciting adrenalin apnea after sinus and aortic denervation (section 3). On the other hand, the occasional occurrence of typical adrenalin

apneas without the reflexes (figs. 5, 6, 7) clearly shows that the phenomenon is not entirely dependent upon them. Although in some cases the blood flow influence was stronger than the reflex one (fig. 12), in other similar experiments the reverse relation existed (fig. 13).

The explanation of these variations lies, I believe, in differences in tone of the finer blood vessels supplying the respiratory center. This factor must be a determining influence in the effectiveness of a given increase in cerebral arterial pressure, for upon it must depend to a very large extent the effectiveness with which stimulant material can be removed from the

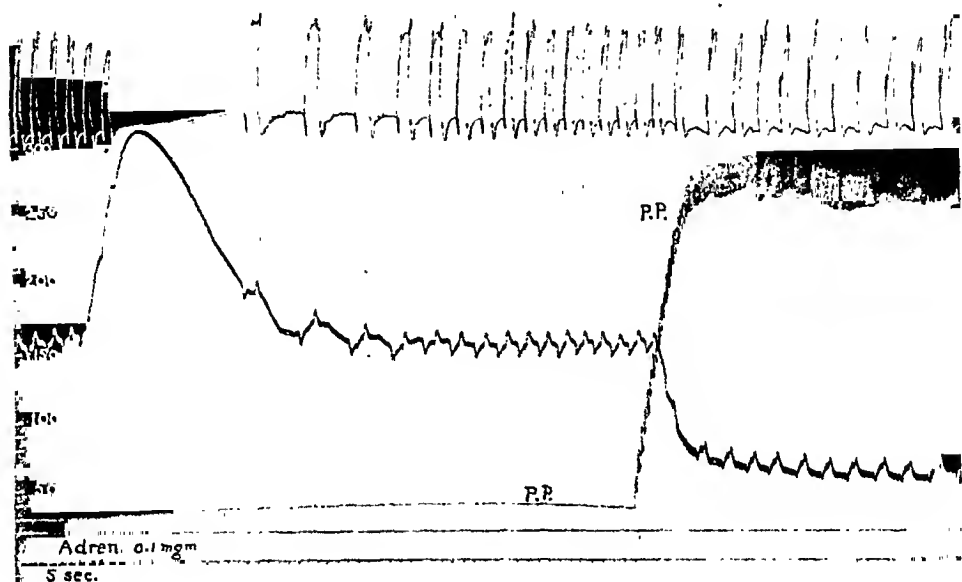


Fig. 12. Comparison of respiratory depressant effects of adrenalin and of high endosinus pressure. Dog—barbital; vagi cut; pneumograph; crossed-perfusion of both sinuses. Adrenalin injected into femoral vein with pump stopped; after recovery, pump started and resistance adjusted so as to bring endosinus pressure to approximately the same level as that reached by systemic pressure after adrenalin.

cells of the center. It is this factor, in my opinion, that accounts for the apnea which occurs when blood is readmitted into the previously occluded vertebral and denervated carotid arteries (figs. 1, 2), or when intracranial pressure is suddenly lowered from a high level (figs. 3, 4); for cerebral vessels are known to dilate during a period of acute anemia (16) (5), and when blood is readmitted into them even at the same pressure as before it must exert an unusually marked influence upon the internal conditions of the cells supplied by them. It is possible that under the circumstances which Heynans regards as normal the cerebral vessels are actually in a state of abnormal constriction, so that the vegetative centers are affected little or

not at all by rise in cerebral arterial pressure. While it cannot be denied that adrenalin apnea in the intact animal is at least partly due to reflexes from the circulation, the fact remains that it can also be elicited without the reflexes. I do not believe that the relative parts played by the reflex and blood flow influences in effects of this sort in the intact animal can be determined, even approximately, until more is known about the intrinsic

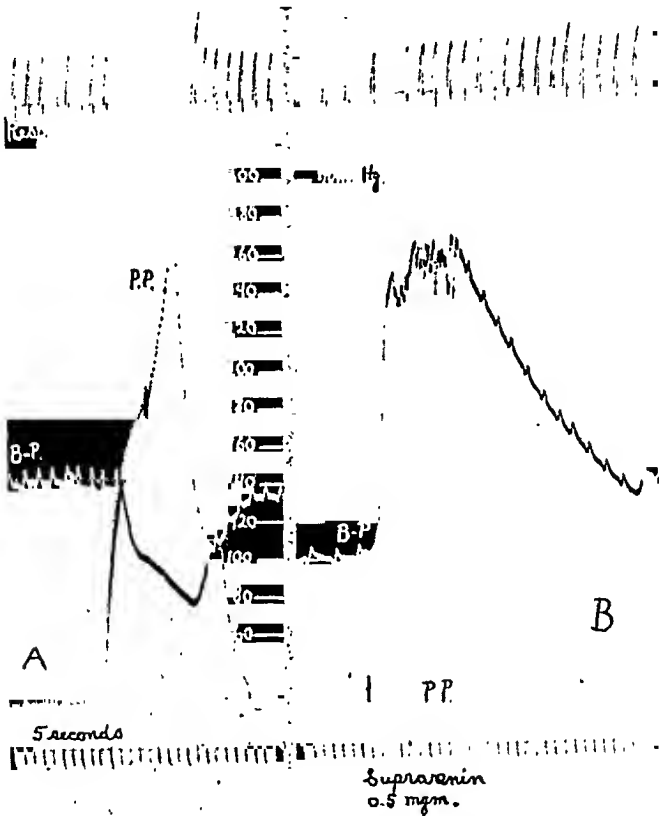


Fig. 13. Same comparison as figure 12. Dog—barbital; vagi cut; pneumograph; sinuses perfused with defibrinated dog blood.

A—effect of elevation of endosinusal pressure to 280 mm. Hg.

B—effect of increase in cerebral blood flow by intravenous injection of suprarenin bitartrate (0.5 mgm.)

regulation of the cerebral circulation. Until then there is no means of defining normal or physiological conditions, and selected examples which prove one point may be countered by others, obtained under other but apparently no more abnormal conditions, which prove the opposite.

There is one other claim, made by Heymans (2) (15), and by Koch (11), which implies that sinus reflexes are necessary to the maintenance of normal respiration in the same sense as they are necessary to the maintenance

of normal blood pressure. It is that the sinus mechanism exercises a tonic inhibitory influence upon the respiratory center. The evidence is the hypernea which follows immediately upon acute section of the sinus nerves or upon occlusion of the innervated common carotid arteries; it is assumed that the latter procedure acts entirely through abolition of tone in the sinus reflex apparatus. Although I have been able to confirm the observations, I believe the above claim should be accepted with considerable reservation, if at all. This is mainly because, even if these hyperpneas are due entirely to removal of a previously exerted inhibitory reflex influence,

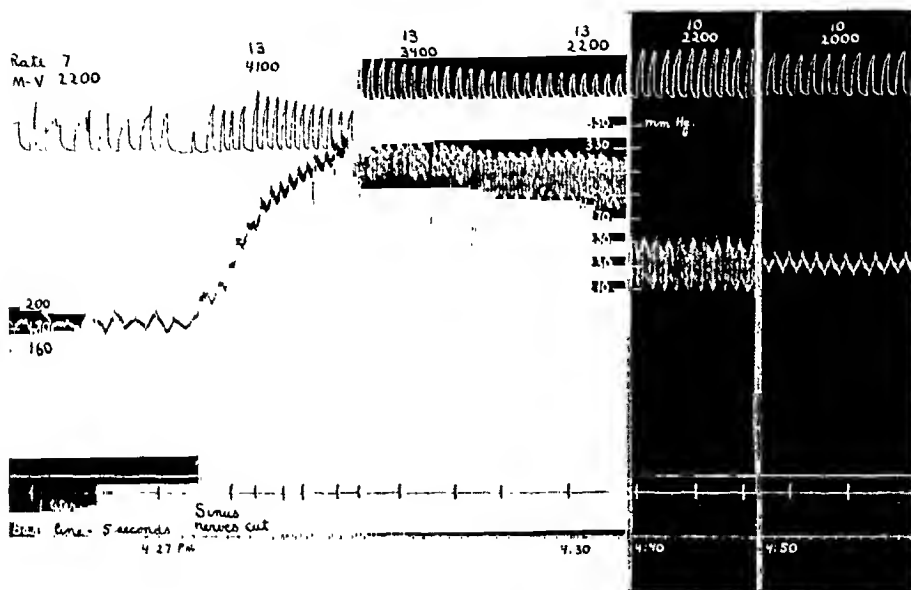


Fig. 14. Respiratory and circulatory effects of acute denervation of sinuses and aorta. Dog—barbital; vagi cut; pneumograph and measurement of expired air, signal showing each liter. At arrow, both sinus nerves were cut: note immediate hyperpnea, with prompt return to same level of ventilation as before. Subsequent tracings follow 10 and 20 minutes later. Sinus denervation permanently removed "apneustic" irregularity of breathing, but did not permanently increase minute-volume.

they are only transitory (1, figs. 1 and 2; fig. 14 of this paper); the hypertension, on the contrary, is much more persistent. It is quite evident, therefore, that tonic inhibition by the sinus reflex mechanism is not nearly as essential to the maintenance of normal breathing as it is to the control of blood pressure: in the case of the respiratory center other mechanisms must be able to assume this function rapidly and completely, which is clearly not the case with the vasomotor and cardio-regulatory centers. It is, however, by no means certain that the hyperpnea produced by section of the sinus nerves or by occlusion of the common carotids is due simply

to removal of inhibitory influences from the sinuses. It was pointed out by Koch (9) that the sinus nerve is a mixed nerve, so that transitory hyperpnea following its section may be due to the inauguration or removal of afferent nerve impulses that had nothing to do with specific sinus reflexes. Furthermore, in all of the recorded examples illustrating this phenomenon blood pressure rose markedly after section of the nerves, so that the condition of the respiratory center might well have been altered in more ways than the simple removal of afferent inhibitory impulses from the sinuses. Likewise in the case of the hyperpnea of carotid occlusion, reasons have already been given (1) for suspecting that it may not always be due simply to inactivation of the sinus reflex apparatus. Since this procedure reduces cerebral blood flow by about 45 per cent (1) it is not unlikely that the effects may be complicated.

For these reasons it seems to me that the discovery of the existence and potency of respiratory reflexes from the carotid sinuses does not alter, in any essential respect, the current conceptions of the mechanism of respiratory regulation. The reflexes seem to be necessary in only one respect, namely, the hyperpnea of anoxemia. Otherwise they accomplish nothing that cannot be accomplished quite as well without them, by alterations in blood supply of the respiratory center. They probably play a part in the respiratory effects of alterations in cephalic blood pressure in the intact animal, but how great or important this part will be must depend to a very large extent upon existing experimental conditions. The state of tone of the blood vessels supplying the respiratory center must be of utmost importance to the blood flow influence, and the sensitivity of the sinus reflex mechanism, peripheral and central, is correspondingly important to the reflex influence. It is quite likely that differences in type and depth of narcosis, trauma, variations in chemical composition of the blood, abolition or inauguration of afferent nerve impulses, etc., all have effects upon one or both of these influences but there is as yet no sufficient evidence to permit even a tentative effort at evaluation.

CONCLUSIONS

1. The respiratory center is shown to be depressed by increase in its blood supply, produced by release of occluded vertebral and carotid arteries, by reduction of high cerebrospinal pressure, by intravenous injection of adrenalin, and by acceleration in cerebral perfusion flow; sinus and aortic reflexes played no part in these effects. In two experiments the vasomotor center was found to behave similarly.

2. It is concluded that the respiratory center is directly affected by alterations in its blood supply in the manner stipulated by the hypothesis of Gesell, namely, because the concentration of chemical stimulant material within its cells is directly influenced thereby.

3. Since the existence and potency of sinus respiratory reflexes, aroused by changes in endosinusal pressure, is established beyond question, the respiratory effects of alterations in cephalic blood pressure may be due either to reflexes, or to alterations in central blood flow, or to both.

4. Reasons are given for believing that under suitable conditions the blood flow influence is quite as sensitive as, and decidedly more powerful than the reflex influence, although the sensitivity of the former is more variable than that of the latter.

5. It is suggested that the effectiveness of the blood flow influence is largely determined by the state of tone in finer medullary blood vessels, and since practically nothing is known about this factor, it is impossible to define normal conditions or to attempt an evaluation of the blood flow influence.

6. It is concluded that sinus respiratory reflexes are essential in only one respect, that being the hyperpnea of anoxemia. Otherwise they accomplish nothing that cannot be accomplished quite as well without them by changes in central blood flow. They are not necessary to the maintenance of normal breathing in the sense that they are necessary to the maintenance of normal blood pressure. The sensitivity of the cells of the center to changes in CO_2 tension or pH of arterial blood is vastly greater than that of the sinus reflex mechanism to any chemical changes in the blood. The central organ therefore retains its position as the most highly specialized part of the nervous mechanism concerned in respiratory control.

BIBLIOGRAPHY

- (1) SCHMIDT, C. F. *This Journal*, 1932, ci, 94.
- (2) HEYMANS, C. AND J. J. BOUCKAERT. *Journ. Physiol.*, 1930, lxi, 254.
- (3) HEYMANS, C. *Arch. Internat. de Pharmacodyn. et de Thérap*, 1929, xxxv, 269.
- (4) HEYMANS, C. *Rev. Belge des Sci. Med.*, 1929, i, 507.
- (5) SCHMIDT, C. F. *This Journal*, 1928, lxxxiv, 202.
- (6) EYSTER, J. A. E. *Journ. Exper. Med.*, 1906, viii, 565.
- (7) WRIGHT, S. *Journ. Physiol.*, 1930, lxi, 493.
- (8) GESELL, R. *This Journal*, 1923, lxvi, 5.
- (9) KOCH, E. *Die reflektorische Selbststeuerung des Kreislaufes*. Dresden, 1931.
- (10) MOISSEJEFF, E. *Zeitschr. f. d. gesamt. exp. Med.*, 1926-27, liii, 696.
- (11) KOCH, E. AND R. E. MARK. *Zeitschr. f. Kreislaufforsch.*, 1931, xxiii, 391.
- (12) GOLLWITZER-MEIER, K. AND H. SCHULTE. *Pflüger's Arch.*, 1931, ccxxix, 251.
- (13) HALDANE, J. S. *Respiration*. New Haven, 1922.
- (14) SCHMIDT, C. F. *This Journal*, 1928, lxxxiv, 242.
- (15) HEYMANS, C., J. J. BOUCKAERT AND L. DAUTREBANDE. *Arch. Internat. de Pharmacodyn. et de Thérap.*, 1930, xxxix, 400.
- (16) WOLFF, H. G. AND H. S. FORBES. *Arch. Neurol. and Psychiat.*, 1928, xx, 1035.
- WOLFF, H. G. AND H. L. BLUMGART. *Ibid.*, 1929, xxi, 795.

QUANTITATIVE COMPARISON OF SOME MUSCLE AND NERVE REACTIONS AFTER DECEREBRATION AND DECAPITATION

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In two previous communications published in THIS JOURNAL the author has shown that the blood and muscle extracts of various animals, when obtained after decerebration and other injuries to the brain *in situ*, have a greater toxicity than similar specimens taken from the same species after arteriotomy or after complete decapitation (1) (2). From the experimental data presented in the papers mentioned above it was argued that the greater toxicity of blood and muscle extracts obtained after injuries to the brain was due to rapid breakdown of the nervous tissue and consequent formation of toxic bodies carried into the blood stream and distributed to various parts of the body before the heart stopped beating and the circulation ceased. These differences in toxicity were demonstrated by special phytopharmacological methods devised by the author for testing blood sera, tissue extracts and various drugs on plant-physiological test objects or living plants and plant tissues (3). Following these observations it was logical to inquire whether a difference in methods of slaughter would reveal a corresponding difference in zoöphysiological and zoöpharmacological experiments. In this paper the results of such experiments on animals are presented.

METHOD OF STUDY. Experiments were performed, for the most part, on frogs and white rats and occasionally on guinea pigs and kittens. The studies on frogs were carried on in two ways. One method was to study the viability of sensory nerve endings in the skin of the legs, as indicated by the activity of the reflex response of the muscles on dipping the feet in weak acid solutions. In every experiment two frogs of the same species, size and physical condition were selected. One of these was decapitated by cutting through the skull with scissors in the region just behind the eyes. The other frog was carefully pithed with a sharp probe so as to destroy that portion of the brain corresponding to the part removed in the decapitated animal. The sensory reflexes of both legs of the two frogs were next tested by dipping the feet in 0.5 per cent of hydrochloric acid.

Observations were then made to determine the quickness of response and the duration of life in the reflex apparatus of each frog.

In another group of frog experiments, the animals were decapitated and decerebrated in the manner described above; and ten to thirty minutes after the operations had been performed, preparations of the gastrocnemius muscle and sciatic nerve were made, and tested with a faradic current at regular intervals to ascertain the activity of nerve and muscle in each frog until no further response could be obtained with the maximum amount of electric energy.

In experiments on rats, two distinct kinds of experiments were also carried out. Large healthy white rats (*Mus norvegicus*) were selected. Some of these were killed by etherizing the animal and completely severing the head. In other rats, the brain tissue was extensively destroyed under ether by piercing the occiput with a sharp probe and stirring it about in the skull cavity.¹ The hearts of the decerebrated, unconscious animals continued to beat for some minutes after the brain was destroyed. In other experiments, the rats were killed by concussion, or a blow on the head: and in still other studies, instead of being decapitated, the animals were etherized and bled to death by cutting through the vessels of the throat. In a number of special experiments, involving neither decapitation nor decerebration, rats were killed by ether, chloroform, or carbon monoxide. After the rats had been killed by these various methods, two sets of studies were made. In one series of experiments, the activity of the sciatic nerve and muscles of the leg was tested at regular intervals with a faradic current, and the quickness and strength of the responses were carefully noted. These observations were continued until the sciatic nerve no longer responded even to the maximum current available. After death of the nerve the response of the skeletal muscle to direct application of the electrodes was observed, and the final death point of the tissues determined by failure to contract even to the maximal electric stimuli available.

In another series of rats, a different kind of experiment was made. In these, decapitation and decerebration were respectively performed in the manner described above. The *vasa deferentia* were then carefully dissected from connective tissue and kept alive in oxygenated Locke's solution maintained at body temperature. The vas deferens of the rat is an excellent organ for the study of the effect of pharmacological agents on smooth muscle. Vas deferens preparations of exactly the same size and length, but obtained from rats killed under different conditions, were suspended in 50 cc. of oxygenated Locke's solution at body temperature and the height of contraction produced by addition of 0.1 cc. of epinephrine hydrochloride to this medium was determined and measured. As will be seen, a distinct

¹ The word *decerebration* is used in this paper to denote any extensive destruction of the brain.

and marked difference in the degree of contraction was noted after decerebration and decapitation, respectively.

RESULTS. I. *Experiments on frogs.* A. *Response of sensory nerve endings.* Frogs were decapitated and decerebrated in the manner described above, and the reflex response of the leg muscle to chemical irritation was tested by dipping the foot in 0.5 per cent hydrochloric acid. The quickness of the reflex withdrawal of the foot from the acid solution was noted and the reaction time measured in seconds. The leg was then rinsed in a beaker of water to remove the chemical irritant. Such tests were repeated at intervals of five or ten minutes until the reflex could no longer be elicited or, in other words, the sensory nerve endings were dead. Duration of life in such preparations was carefully noted. In most of the experiments performed it was found that the reflexes died much sooner in the decapitated than in the decerebrated frogs. Thus, for instance, in one experiment the decerebrated frog continued to respond after five hours while the decapitated frog was dead in four hours. In another experiment the decerebrated animal lived three hours while the decapitated frog died in one and a half hours. While the explanation for this difference in reaction between decerebrated and decapitated frogs is not wholly clear, it certainly cannot be altogether due to the loss of blood in the latter because pithing the frogs for decerebration produced nearly as much bleeding as did cutting across the skull.

B. *Experiments on nerve-muscle preparations.* Nerve-muscle preparations of the sciatic nerve and gastrocnemius were taken from both decapitated and decerebrated frogs and kept from desiccating by applications of physiological saline solution. The response of the sciatic nerve to the electric current in each case was then tested with a Harvard inductorium, and the least energy required to produce a definite response in the nerve-muscle preparations was ascertained every five or ten minutes by adjusting the position of the secondary coil until death of the nerve. The time interval from the beginning of the experiment to this point was carefully noted. Thereafter the skeletal muscle still retained its contractility to direct stimulation by the electric current; and the gastrocnemius was tested by application of the electrodes at frequent intervals until the muscle no longer responded to the maximal stimuli available.

In the majority of such experiments it was found that the duration of life, as tested by the faradic current, was much longer in the nerve-muscle preparations taken from decerebrated frogs than in those obtained from decapitated specimens. The sciatic nerve usually lived from one to two hours longer in the former than in the latter. This difference between decerebrated and decapitated animals was even more strikingly exhibited by experiments on rats.

II. *Experiments on rats.* A. *Nerve-muscle preparations of rats' legs.*

A large number of rats were killed in different ways and immediately after death the sciatic nerve of one leg (or both) was dissected from the connective tissue and tested for its response to the faradic current. Some of the rats were decapitated; others were decerebrated in the manner described above. Still other rats were killed by severing the vessels of the throat; and in a few experiments the animals were asphyxiated by ether, chloroform or carbon monoxide. The minimal intensity of the faradic current from a Harvard inductorium required to produce a contraction of the leg muscles on application to the sciatic nerve was ascertained and expressed by the distance in centimeters of the secondary from the primary coil. This was repeated every five or ten minutes until the sciatic nerve failed to respond. The electrodes were then applied directly to the skeletal muscles of the leg, and these were likewise tested at regular intervals until they failed to respond to the maximum stimulus available.

A very marked difference was noted in the viability of the nerve and muscle preparations in relation to the method of slaughter. In general,

TABLE 1

DECAPITATED RATS	TIME	DECEREBRATED RATS	TIME
	<i>minutes</i>		<i>minutes</i>
Life of sciatic nerve (average of 21 experiments).....	52	Life of sciatic nerve (average of 23 experiments).....	91
Life of gastrocnemius (average of 20 experiments).....	97	Life of gastrocnemius (average of 20 experiments).....	123

the sciatic nerve and muscles of the leg lived longer in decerebrated rats than in those which had been decapitated or killed by bleeding or asphyxiation. Thus, for instance, in one experiment, the nerve of a rat, weighing 260 grams and decapitated under ether, continued to respond to electric stimulation for 55 minutes, while the muscle continued to respond for 110 minutes. In another rat of the same weight, decerebrated under ether at the same time, the nerve lived for 110 minutes and the muscle for 130 minutes after death. Table 1 shows the difference between the average figures obtained from all the decapitation and decerebration experiments performed in this connection. The duration of life of both nerve and muscle from animals killed by arteriotomy, or cutting the vessels of the throat, was practically the same as that in preparations from decapitated rats. Thus, for instance, in one experiment after arteriotomy the nerve lived for 60 minutes while the muscles of the leg ceased to respond after 90 minutes. The same was true of preparations excised after death by ether or chloroform. Carbon monoxide, however, produced more rapid death of both nerve and muscle, as tested by the faradic current.

C. Experiments on the vas deferens. The author has used the vas deferens

of the rat extensively for pharmacological testing of the effects of drug and tissue extracts on smooth muscle organs. Thus, Macht and Matsumoto (4) found that this organ is particularly sensitive to extracts of corpus luteum and the degree of contraction produced by such solutions on the vas deferens was an index to the physiological activity of the hormone, running parallel to the results obtained by the vaginal smear method (5). The vas deferens gives a prompt and marked contraction on treatment with epinephrine.

About two hundred experiments were performed on vasa deferentia obtained from rats killed by different methods. The results of these studies indicate a distinct difference in the degree of contraction produced by epinephrine on vasa deferentia from decerebrated rats and those obtained from decapitated animals. When carefully dissected and tested as to their response to epinephrine, the two vasa deferentia from any healthy rat were ordinarily found to give nearly the same height of contraction. Again, when any one vas preparation was tested with epinephrine and then washed thoroughly with Locke's solution, it could be contracted to the same degree by one, two and even three similar successive doses of the drug administered at short intervals of time.

Studies were made on the response of the vasa deferentia obtained from decapitated versus decerebrated rats; and it was found that in 90 per cent of all the experiments made, the contraction elicited by 0.1 cc. of epinephrine was much greater in vasa taken from decerebrated rats than in those removed from decapitated animals. In 10 per cent of the experiments there was either no difference in the degree of stimulation by epinephrine or, in a few cases, the specimen from the decerebrated animal responded very poorly to the drug. The following figures show the average height of contraction of vasa deferentia taken from decapitated and decerebrated animals, respectively.

	<i>mm.</i>
Average height of contraction by epinephrine of 50 vasa deferentia from decapitated animals.....	55
Average height of contraction by epinephrine of 50 vasa deferentia from decerebrated animals.....	87

The effect of arteriotomy, or bleeding without decapitation, was the same as that obtained after complete decapitation of the rats. In all the experiments the organs were lightly weighted so as to obtain the maximum contraction with a lever the short arm of which was 3 cm., while the long arm was 33 cm. In these comparative studies care was taken to make the vas deferens preparations of the same length before beginning the experiments, as measured at room temperature.

COMMENT. The data adduced above, together with the results obtained in many other experiments performed by the author, indicate that the

method of slaughter exerts a definite influence on the reactions of surviving nerve and muscle preparations. This was found to be the case with the sensory nerve endings of the frog's skin, the sciatic nerve and gastrocnemius muscles of both frogs and rats, and with the smooth muscle of the vas deferens of the rat. It was found in each case that an extensive destruction of or injury to the brain prolonged the viability of those organs or tissues, as compared with the duration of life of the same tissues taken from decapitated or exsanguinated animals. The reason for this phenomenon is not altogether clear. It may be partly due to the greater loss of blood in the decapitated animals but the most obvious hypothesis suggesting itself is that the destruction of brain tissue leads to the formation of some products of decomposition which the blood carries around the body before the heart has come to a standstill. Thus, for instance, in case of the vas deferens, such abnormal products of decomposition may be regarded as sensitizing the organ to the effects of epinephrine. This view is supported by the findings obtained from experiments on rats, in which one vas deferens was removed under ether anesthesia, the brain being afterward destroyed *in situ*, and the other organ excised ten minutes later. It was interesting, furthermore, to note that small quantities of thyroxin added to the physiological solution in which the vasa deferentia were suspended sensitized those organs to a subsequent dose of epinephrine; and such sensitization by thyroxin occurred in organs from both decapitated and decerebrated animals. The "decerebrated" specimens, however, were not sensitized by the thyroxin to the same degree as the "decapitated" vasa deferentia. While most of the experiments in the present investigation were performed on vasa deferentia, it is plausible that other surviving smooth muscle organs may also show similar differences in contractility in relation to the method of slaughter. In fact, a number of experiments performed by the writer indicate that the uteri of guinea pigs reveal differences analogous to those of the vasa deferentia from decapitated and decerebrated rats. These findings, of course, are of practical importance in connection with the physiological and pharmacological assay of various drugs. Thus, for instance, in the standardization of preparations of the posterior lobe Roth (6) states that the best results are obtained with virgin guinea pigs which are killed by rapid decapitation. Again, Clemen (7) states that the material used in preparation of thromboplastin is obtained from kohshered animals killed by arteriotomy, without stunning, because the stunning of cattle injures the brain tissues, rendering them unfit for the manufacture of this product.

SUMMARY

1. The method of slaughter of certain laboratory animals produces a difference in physiological and pharmacological response of certain surviving organs and tissues.

2. The sensory nerve endings of the frog's skin retain their vitality for a longer period of time in decerebrated than in decapitated animals.

3. Nerve-muscle preparations of the sciatic and gastrocnemius cease to respond sooner to electric stimulation after decapitation than after decerebration or extensive injury to the brain.

4. Surviving vasa deferentia dissected from rats after injuries to the brain respond more powerfully to epinephrine than preparations excised from decapitated or exsanguinated animals.

BIBLIOGRAPHY

- (1) MACHT. This Journal, 1931, xcvi, 662.
- (2) MACHT AND COOK. This Journal, 1931, xcvi, 662.
- (3) MACHT. Science, 1930, lxxi, 302.
- (4) MACHT AND MATSUMOTO. Journ. Urol., 1919, iii, 63.
- (5) MACHT, STICKELS AND SECKINGER. This Journal, 1929, lxxxviii, 65.
- (6) ROTH. U. S. Public Health Service, Hygienic Lab. Bull., 1914, c, 20.
- (7) CLEMEN. By-products in the packing industry. Univ. Chicago Press, 1927, p. 229.

THE EFFECT OF METHYLENE BLUE ON HCN AND CO POISONING¹

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It has long been known that methylene blue increases certain biological oxidations. Meyerhof (1912) found that the O consumption of yeast was raised 200 to 300 per cent above normal by methylene blue. It has been demonstrated by Heymans and Heymans (1922) that a rise in temperature took place in dogs that had received methylene blue injections. They suggest that the effect is probably one of a catalyzer upon the oxidative processes.

More recently Barron (1929) and Barron and Harrop (1928) concluded from experiments on eggs of sea urchins and star-fish, erythrocytes, and mammalian tissues that methylene blue acts as a catalyst in activating O. Furthermore, they found that methylene blue has no effect on increasing the O consumption of those tissues which do not possess aerobic glycolysis, and that inhibition of the respiratory ferment by CN affects in no way the oxidation power of methylene blue. In this connection Thunberg (1918) found that CN does not hinder decoloration of the dye in the system, succinic acid-enzyme-methylene blue, while it does hinder the uptake of O in the system succinic acid-enzyme-O. Wendel (1931) demonstrated that buffered cyanide markedly increases the rate of oxidation of lactate to pyruvate by red blood cells in the presence of methylene blue.

In view of these observations it was thought desirable to see whether such an effect of methylene blue could be demonstrated in living mammals whose aerobic respiration had been interfered with.

Since Warburg (1910, 1923, 1926) has long demonstrated that both CN and CO are specific respiratory poisons, these two gases were used to inhibit aerobic respiration. Warburg (1930) has furthermore shown that the O uptake of red blood suspensions is practically unaffected by CO in the presence of sufficiently large amounts of methylene blue.

It had already been shown empirically in laboratory animals by Sahlin (1926), Eddy (1931) and other workers that methylene blue could be used to

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antagonize the effects of NaCN by injecting it intraperitoneally either before the NaCN injections or immediately afterwards. No work has been done to the writer's knowledge on antagonizing by methylene blue the poisonous effects of inhalation of HCN or CO gases.

For these experiments 80 rats were used. They were divided into groups of 20 each, using that number for each set of control experiments with each gas and for the methylene blue-treated animals with each gas.

The animals were placed one at a time in a chamber containing HCN gas (evolved from the decomposition of NaCN) or CO (about 1 per cent by volume in air, produced by the action of hot H_2SO_4 upon formic acid).

TABLE I

Average of 80 experiments (20 in each group) showing time during which HCN or CO was administered and time of recovery of control rats and those treated with methylene blue injections

A and C, controls; B and D, treated

CONTROLS		METHYLENE BLUE TREATED	
A	HCN	B	
In HCN 3.3 minutes	Recovery 11.2 minutes (100%)	In HCN 3.3 minutes	Recovery 4.0 minutes (36%)
Variation 1 to 7 minutes	Variation 5 to 25 minutes	Variation 1 to 6½ minutes	Variation ¾ to 9½ minutes
C	CO	D	
In CO 1.1 minute	Recovery 7.5 minutes (100%)	In CO 1.1 minute	Recovery 4.3 minutes (57%)
Variation 25 seconds to 2½ minutes	Variation 4½ to 12 minutes	Variation 25 seconds to 3½ minutes	Variation ¾ to 7¼ minutes

The gas in the chamber was renewed after each experiment and control. The time elapsing before unconsciousness set in was noted. As soon as an animal became unconscious and gave no further reactions to external stimuli, it was quickly removed from the chamber, and given an intraperitoneal injection of methylene blue. The time of recovery was then determined, using as a criterion of recovery the ability of the animal to run straight forward, since it was found that the use of the hind legs was delayed even when the animal was able to sit up.

The amount of methylene blue injected was 1 cc. of 0.01 M solution in Ringer-glucose solution for each 100 grams of body weight. The results for both HCN and CO are given in table 1.

These results show that the recovery of animals having received methyl-

ene blue is considerably accelerated as compared with the controls. The group averages show that whereas the control and experimental animals lost consciousness in equal times, the times of recovery (taking that for the controls in each case as 100 per cent) were 36 per cent for HCN and 57 per cent for CO. Ringer-glucose injections alone had no effect on the time of recovery. When methylene blue was injected subcutaneously no increase in the rate of recovery was observed indicating that rapid diffusion of the dye was necessary to produce the desired effect.

It would seem therefore from these results that intraperitoneal injections of methylene blue increased certain biological oxidations so that the animals were enabled to recover in a shorter time. The results are consistent with the observations on other materials showing an antagonism between methylene blue and CN or CO. These experiments, however, throw no light upon the question of interpretation of the catalyzing ability of methylene blue, i.e., whether it acts as a catalyst in oxidizing bivalent Fe to trivalent Fe according to Warburg, Kalowitz and Christian (1930), or whether according to Barron and Hoffman (1930) it increases the oxidation of some of the decomposition products of carbohydrate metabolism.

SUMMARY

If rats are made unconscious by inhalation of gaseous HCN or CO, their rate of recovery can be considerably accelerated by intraperitoneal injections of methylene blue. Recovery in the case of HCN required 36 per cent and in that of CO, 57 per cent of the time required for recovery of the corresponding controls.

BIBLIOGRAPHY

- BARRON, E. S. G. 1929. *Journ. Biol. Chem.*, lxxxi, 445.
BARRON, E. S. G. AND G. A. HARROP. 1928. *Journ. Biol. Chem.*, lxxix, 65.
BARRON, E. S. G. AND L. A. HOFFMAN. 1930. *Journ. Gen. Physiol.*, xiii, 483.
EDDY, N. B. 1931. *Journ. Pharm. Exper. Therap.*, xli, 449.
HEYMANS, J. F. AND C. HEYMANS. 1922. *Arch. Intern. pharmacodynamie*, xxvi, 443.
MEYERHOF, O. 1912. *Pflüger's Arch.*, cxlix, 250.
SAHLIN, B. 1926. *Skand. Arch. f. Physiol.*, xlvii, 284.
THUNBERG, T. 1918. *Skand. Arch. f. Physiol.*, xxv, 163.
WARBURG, O. 1910. *Zeitschr. physiol. Chem.*, lxvi, 305.
1923. *Biochem. Zeitschr.*, cxlii, 518.
1926. *Biochem. Zeitschr.*, clxxvii, 471.
WARBURG, O., F. KALOWITZ AND W. CHRISTIAN. 1930. *Biochem. Zeitschr.*, ccxxvii, 245.
WENDEL, W. B. 1931. *Journ. Biol. Chem.*, xcii, xlvii.

STUDIES OF THE LIVER FUNCTION OF DOGS

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The advancement in the study of liver function by the use of dyes has been rapid. Abel and Rowntree (1909) observed that phenoltetrachlorophthalein, injected into the blood, appeared in the bile. Rowntree, Hurwitz and Bloomfield (1913) developed a functional liver test, involving the stool collection of phenoltetraehlorophthalein. Whipple, Peightal and Clark (1913) demonstrated that the intensity of hepatic injury, resulting from chloroform, hydrazine or phosphorus, was inversely proportional to the rate of elimination of phenoltetraehlorophthalein by the liver. McNeil (1916) introduced the use of the duodenal tube for the quantitative study of the excretion of phenoltetraehlorophthalein by the liver. Aaron, Beck and Schneider (1921) adopted a stable preparation of phenoltetrachlorophthalein and modified McNeil's duodenal technique, to study liver function.

Recent investigations (Rosenthal, 1922; Rosenthal and White, 1924; Rosenthal and Bourne, 1928; Rosenthal, 1930; and Rosenthal and Lillie, 1931) have emphasized two points: 1, that the determination, in the blood, of the dye which is excreted by the liver, is a valuable index of hepatic function, and also 2, that bromsulphalein is the most acceptable dye available for the physiological study of the liver.

The bromsulphthalein test for liver function is supported by experimental and clinical evidence. Most of the experimental work with bromsulphalein, as an index of hepatic activity, has been carried out with rabbits. However, Rosenthal et al., (1928, 1930, 1931) determined some effects of anesthetics, alcohol, carbon tetrachloride and fat on the hepatic function of dogs.

In attempting to determine the relation between 1, basal metabolism; 2, calcium and phosphorus content of the blood, and 3, liver function; in both hypo- and hyper-thyroid conditions of dogs, we have observed hepatic function in dogs with normal and damaged livers.

METHODS. The Rosenthal bromsulphalein method was used to test the liver function of dogs in this series of experiments. The bromsulphalein and the corresponding set of color standards were obtained from Hynson, Westcott and Dunning, Baltimore.

Normal dogs. Eleven dogs were used for the normal tests. Two milligrams of dye per kilo body weight were injected into the saphenous vein of the dog tested. After injection, a 4 cc. sample of blood was obtained from the saphenous vein of the opposite leg, with a syringe containing dry sodium oxalate. Samples were taken each minute during a period of ten

TABLE 1

The percentage retention of 2 mgm./kgm. of bromsulphalein in the blood of dogs

NORMAL DOGS											CHLOROFORMED DOGS			
Serial number	Minutes after injection										Serial number	Minutes after injection		
	1	2	3	4	5	6	7	8	9	10		10	20	40
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	After 1 day			
2				30		10					14	90	80	
3	100		90	25				20			21	90	70	
4		70	35		15		5	3			23	95	90	
14		80		50	35		12		10					
21	35		25			15		8		5				
1	80		45	35	25	12		8						
9	55	40		10	10	9		5						
10	45				10	5			3					
13	90	50	40	20	12		7							
15	50		30				8	3						
11	60	50	40	30	20	10	5	5	0	0				
Ave.	64	58	44	29	18	10	7	7	4	3				
DOGS WITH HEPATIC CIRCULATION DESTROYED														
Serial number	Minutes after injection							After 10 days						
	5	10	15	20	25	30	35	per cent	per cent	per cent	per cent	per cent	per cent	per cent
9	100	100	99	95	95	93	90	14	15	5				
22	95	95	95	90	90	90	90	21	15	10				
								23	15	5				
Ave.	98	98	97	93	93	92	90	Ave.	15	7				

minutes. The samples of blood were then centrifuged and 1.0 cc. of the plasma was divided equally into two small test tubes. Two drops of 10 per cent NaOH was added to one of the test tubes. Two drops of 5 per cent HCl was added to the other test tube. The percentages of the dye in the plasma were read in a bromsulphalein comparator box.

Dogs with hepatic circulation destroyed. Two dogs with normal liver

action, as shown by the bromsulphalein test, were selected. The dogs were anesthetized with ether and injected with 0.1 gram of heparin. A glass T-cannula was used to anastomose the portal vein and inferior vena cava.

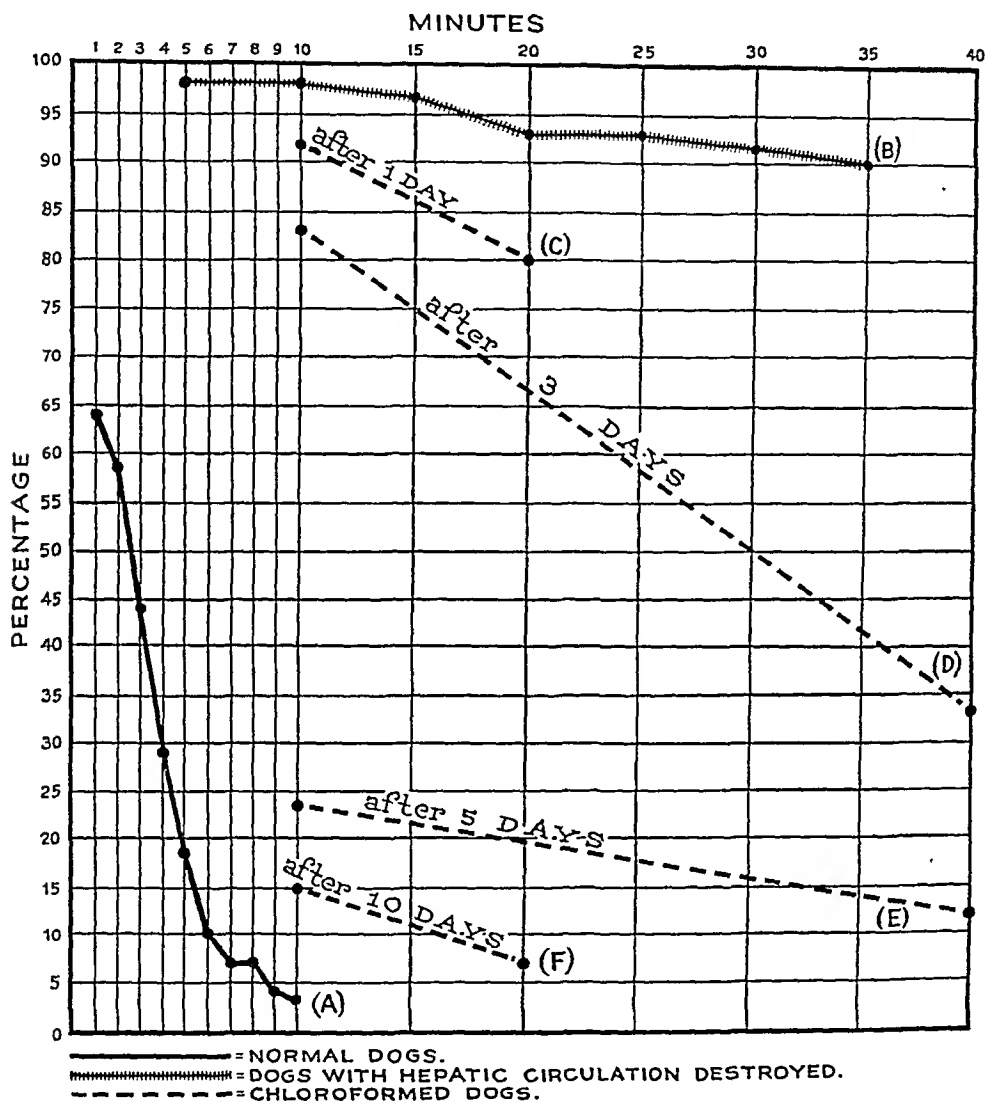


Fig. 1. The physiological action of the liver as indicated by the retention of bromsulphalein in the blood of dogs. The percentage retention of the dye is represented on the ordinates. The minutes, during which the blood samples were taken, are located on the abscissae. The curves show, graphically, the average percentage retention of bromsulphalein recorded in table 1. The letters A, B, etc., are used for reference in the text.

The hepatic blood vessels were ligated. Samples of blood, to be observed for dye retention, were obtained 5, 10, 15, 20, 25, 30 and 35 minutes after the introduction of the bromsulphalein.

Chloroformed dogs. Three dogs were selected and their normal conditions established. Chloroform anesthesia was then administered for a period of two hours and fifteen minutes. The samples of blood were taken at 10, 20 and 40 minute intervals, following the injection of the dye (see table 1).

The percentages of the dye in the plasma of all the animals were determined by the same method.

RESULTS. Bromsulphalein disappeared rapidly from the blood of all normal dogs. The average retention of the dye in normal dogs, as shown in table 1, was 64 per cent the first minute, and 3 per cent the tenth minute, after the injection of the bromsulphalein. The average percentage retention of the dye in the blood of normal dogs, plotted in figure 1, (A), shows a regular decline during the ten-minute observation.

The removal of the dye from the blood of the dogs, after the destruction of the hepatic circulation, was negligible (B, fig. 1). The dye, which normally would have been removed almost completely within 10 minutes, averaged 90 per cent retention in the blood of the anastomosed dogs, 35 minutes after the injection of the bromsulphalein.

Chloroform administration caused a pronounced retention of the bromsulphalein in the blood of the dogs. Curves C, D, E and F, in figure 1 represent the comparative rates of the disappearance of the dye from the blood of the dogs, after the 1-day, 3-day, 5-day and 10-day rest periods. The highest retention of the dye, in the blood of the chloroformed dogs, was observed the first day after the exposure to the anesthetic (table 1). Ten days after the chloroform injury of the liver, however, only 15 per cent of the dye was retained, in the blood of the dogs, 10 minutes after the injection of the bromsulphalein.

DISCUSSION. Ether anesthesia was used in preparation of the dogs for chloroform administration and in those dogs whose hepatic circulation was destroyed. Slight impairment to the liver function may be attributed to the ether. Rosenthal and Bourne (1928) recorded that, after two hours of ether administration, the 15 minute retention of the bromsulphalein in dogs was about 15 per cent.

Rosenthal and White (1925), by the ligation method, proved that the quantity of the bromsulphalein excreted from the blood of rabbits was dependent upon the amount of functional liver as determined by weight. We demonstrated, by joining the portal vein with the inferior vena cava and ligating all of the hepatic blood vessels in the dogs, that, in dogs without any functional liver, practically none of the bromsulphalein introduced into the blood would be eliminated.

In our work, we observed a relationship of the damaged livers of dogs to the detoxication of nembutal (the nembutal was supplied, complimentary, by Abbott, Chicago). We devocalized some dogs, including two of the chloroformed dogs. The chloroformed dogs, when anesthetized with

nembutal, remained in deep anesthesia, 24 and 36 hours, respectively. The normal dogs recovered from the effects of the nembutal in 2 to 3 hours.

The function of the liver in the detoxication of such substances as nembutal, by normal and damaged livers, offers an important problem for investigation.

SUMMARY

The high retention of the dye in the dogs, after the complete blocking of the hepatic circulation and the union of the portal vein with the inferior vena cava, is further proof that the bromsulphalein injected into the blood is eliminated, specifically, by the liver.

The determination of the removal of the bromsulphalein from the blood of dogs, under normal conditions as well as during chloroform and circulatory injury of the liver, indicates that the bromsulphalein test is a delicate and effective method for the study of hepatic function. Samples of blood obtained 3 and 10 minutes after the injection of the bromsulphalein in dogs provide a suitable basis for the physiological study of the liver.

Dogs which received prolonged chloroform anesthesia and, subsequently, were anesthetized with nembutal, remained in deep anesthesia for 24 to 36 hours; while dogs with undamaged livers recovered from the effects of nembutal in 2 to 3 hours.

We appreciate the interest and helpful criticism of Dr. Edward C. Mason.

BIBLIOGRAPHY

- AARON, A. H., E. C. BECK AND H. C. SCHNEIDER. 1921. *Journ. Amer. Med. Assoc.*, lxxvii, 1631.
ABEL, J. J. AND L. G. ROWNTREE. 1909. *Journ. Pharm. Exp. Therap.*, i, 231.
MCNEIL, H. L. 1916. *Journ. Lab. Clin. Med.*, i, 822.
ROSENTHAL, S. M. 1922. *Journ. Pharm. Exp. Therap.*, xix, 385.
1930. *Ibid.*, xxxviii, 291.
ROSENTHAL, S. M. AND W. BOURNE. 1928. *Journ. Amer. Med. Assoc.*, xc, 377.
ROSENTHAL, S. M. AND R. D. LILLIE. 1931. *This Journal*, xcvii, 131.
ROSENTHAL, S. M. AND E. C. WHITE. 1925. *Journ. Pharm. Exp. Therap.*, xxiv, 265.
ROWNTREE, L. G., S. H. HURWITZ AND A. L. BLOOMFIELD. 1913. *Bull. Johns Hopkins Hosp.*, xxiv, 327.
WHIPPLE, G. H., T. C. PEIGHTAL AND A. H. CLARK. 1913. *Ibid.*, xxiv, 343.

THE CHEMISTRY OF EMBRYONIC GROWTH

III. A BIOCHEMICAL STUDY OF THE EMBRYONIC GROWTH OF THE PIG WITH SPECIAL REFERENCE TO NITROGENOUS COMPOUNDS¹

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The field of embryonic chemistry offers an unparalleled opportunity for the study of life processes. The orderly development of a single cell into a highly differentiated multicellular and multiorganned individual involves a series of chemical syntheses and chemical changes which must eventually be understood before we can truly say that we are familiar with the processes that characterize living matter. The embryologists tell us that the morphological changes which occur during embryonic development represent a recapitulation of the history of the race; may it not well be that by studying the sequence of biochemical changes during embryonic growth we will learn something of the biochemistry of evolutionary processes?

Of the many elements which enter into the composition of the embryo, there is one, nitrogen, that can be readily estimated with a fair degree of precision. Furthermore, by making use of certain characteristic reactions we can determine some of the different chemical groupings and compounds which contain this nitrogen, and these compounds in turn are, in the main, constituents of the proteins, which constitute the bulk of the cellular material. We have accordingly devoted our investigation to a comparative study of the forms of nitrogen in the pig embryo at various stages of its development.

HISTORICAL. The first two papers in this series (Gortner (1913, 1914)) dealt with a study of nitrogen changes in the developing eggs of the brook trout, *Salvelinus fontinalis* L., and of the giant salamander, *Cryptobranchus alleganiensis*. It was shown that while there was a loss of weight during development, no nitrogen was lost from the eggs although shifts in the various nitrogen ratios did occur. The basic nitrogen increased at the expense of the mono-amino nitrogen and it was suggested that a part of

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the energy of development was derived from the changes taking place in the protein fractions. A definite synthesis of fat from protein was likewise observed.

These early papers included a brief literature review. After the present study had been completed and, together with an extensive literature review, was largely in manuscript form, the recent comprehensive monograph by Needham (1931) on *Chemical Embryology* became available. This monograph is so complete and so excellently written that the reader may be referred to it for the historical treatment.

Suffice it to say that except for a few papers dealing with the stage of embryonic growth at which enzymatic activity becomes apparent, there are very few papers dealing with the biochemistry of the developing mammalian embryo, and still fewer dealing with its nitrogenous constituents. Almost the only papers in this field are those by Buglia and Costantino (1912). These authors studied the nitrogen fractions in the musculature of cattle embryos at various stages of development in comparison with similar fractions in the adult animal. They find that as growth and development progresses there is an increase in the percentage of nitrogen in the tissues (11.31 to 15.05 per cent), a decrease in the "ammonia nitrogen" (20.40 per cent to 9.14 per cent), an increase in the "formol titratable" nitrogen (49.86 per cent to 66.81 per cent), and, within this fraction, a relative greater increase of the diamino acids than of the monoamino acids. They further show that in the aqueous extracts of the embryonic musculature the monoamino acids predominate (90 per cent) whereas similar extracts of adult musculature are characterized by a predominance (76 per cent) of the diamino acids.

EXPERIMENTAL. *The problem.* A review of the literature disclosed the following points:

1. That no systematic study has ever been made on the mammalian embryo, particularly of the nitrogenous compounds.

2. That no data have been compiled on related studies of incubating hen eggs and developing mammalian embryos, and therefore no fundamental facts exist upon which to base the idea that knowledge obtained in working with the chick embryo, the most convenient system, can be transferred to mammalian development.

3. That the work on nitrogen distribution in chick embryos has been quite confusing. In specific cases the whole egg, including the shell, has been used, while in most instances the entire contents which evidently contained a certain amount of food material in the yolk sac and the excretions of the embryo were employed. These quite naturally gave varied results.

4. The unreliability of the results heretofore obtained can be demonstrated in the two papers of Calvery (1929), in the first of which arginine is reported as at first decreasing and later increasing while in the later publi-

cation it is reported as not changing, while the histidine and lysine results were admittedly undependable.

5. That in spite of the large amount of work done in embryonic chemistry, there is general harmony on only a few points.

We have felt, therefore, that by collecting mammalian embryos at different stages, completely dissecting them free of their membranes, measuring carefully their lengths and classifying them accordingly, we could arrive at some conclusions, which would at least represent only the changes which are taking place within the embryo itself, and might assist in clarifying the literature.

The material. We have chosen to work with pig embryos. In the pig, the duration of gestation ranges for 109 to 123 days (4-4½ months). Usually all the normal embryos of the litter are all in the same phase of development, but however it is a matter of common experience that one does find occasionally embryos that are definitely below or above the average length and are in an entirely different stage. This, however, is the exception rather than the rule, and it can be stated with very few reservations that embryos of the same litter, and embryos the same length from different litters, are in the same phase of embryonic life. The average duration of development from the moment of fertilization until all the parts or foundations of the embryo have come into existence is between 28 to 30 days and represented by the 12 to 15 mm. embryo. This period has been termed the critical period. From this, one can easily see that the total duration of gestation can be divided into four critical periods,—each approximately one month.

METHODS. The embryos, ranging in length from 6 mm. to 240 mm., were collected within ten minutes after the death of the mother. In most cases the specimens were still alive, as evidenced by the continuation of the fetal heart beat. Embryos of 60 mm. or less were dissected from the uterine attachments without rupture of the fetal membranes, while larger specimens were kept under amniotic fluid. This precaution was taken to prevent any possibility of drying before the material could be weighed.

Insofar as was possible, the collections were removed from the amniotic fluid within an hour, dried with filter paper, weighed, and immediately placed in acetone for dehydration. The acetone was changed every 12 hours. In order to insure the immediate access of the dehydrating agent to all parts of the larger embryos, and thus prevent autolysis, the abdominal and cranial cavities were incised, care being taken to avoid the blood vessels.

After 72 hours of this preliminary acetone treatment, the material was ground, and, in a continuous extraction apparatus, further submitted to acetone for an additional 48 hours, at the end of which time it was dried at a temperature of 65°C. for ½ hour and weighed. This weight was tabu-

lated as the "dry weight." An obvious objection to this procedure is based on the fact that acetone, particularly when hot, will extract some lipids. The amount of the latter removed, however, is negligible. After this weighing, the material was extracted for 48 hours with alcohol and anhydrous ether successively, dried again at 65°C., for $\frac{1}{2}$ hour and ground in a porcelain ball mill.

The method used of determining the nitrogen distribution is the Van Slyke (1910) method for the determination of chemical groups characteristic of various amino acids, with certain modifications, as recommended by Plimmer and Rosedale (1925), and Cavett (1932), together with certain minor changes with regard to dilution and apparatus as was suitable to this particular problem. The material was hydrolyzed for 24 hours with 20 per cent HCl.

From the beginning we were fully aware that this powdered embryonic material was not a pure protein, and that it must contain certain inorganic salts, carbohydrates, purine bases and other nitrogenous compounds. We were also cognizant of the fact that the Van Slyke nitrogen distribution was reliable for only pure proteins. Attention was called to this by no other than Van Slyke himself. He stated that the method was designed for use only with proteins not accompanied by other classes of substances, particularly nitrogenous substances, which would obviously falsify the interpretation of the results unless the behavior of the non-protein substances were so accurately known that the proper corrections could be made. Just as a matter of emphasis it might be mentioned that Gortner and Blish (1915) have shown that the presence of carbohydrates under the conditions used for protein hydrolysis (20 per cent HCl) will definitely effect the amount of tryptophane converted into humin. More recently, Hauge (1931) pointed out that the presence of fats, particularly the glycerol radical, increases the acid soluble humin, decreases the amino nitrogen in the basic and non-basic fractions and decreases the arginine nitrogen. Any amines formed during acid hydrolysis will appear in the ammonia fraction. Some of the purines and other nitrogenous substances, such as creatin, that occur in animal tissues, are no doubt broken down into simpler compounds. We have shown in preliminary experimentation that creatin and creatinine, under the conditions used in determining arginine, will contribute quantitatively to the ammonia which is formed. It will be noticed subsequently that certain results are listed in the tables under "arginine," "histidine" and "lysine." Obviously these substances are not pure arginine, histidine or lysine, but merely contain those nitrogen fractions which correspond in their chemical characteristics to the distribution of the arginine, histidine and lysine of pure proteins.

In agreement with the above citations, it might be objected that these other substances present besides the proteins would invalidate the results.

These substances, however, are probably present in all the samples, and inasmuch as all samples were treated under exactly the same rigid procedures, we feel that we are justified in concluding that the results we have obtained are comparable.

Tyrosine was determined by the method of Folin and Marenzi (1929), hydrolyzing the material with 20 per cent NaOH and using either the phenol reagent or sodium nitrite solution to develop the color reaction.

Glutathione was determined by the method proposed by Tunnicliffe (1925). One part of fresh tissue was extracted with 4 parts of 10 per cent trichloroacetic acid, the final solution was brought to a convenient volume

TABLE 1

Showing the average weight and the per cent of water, ash, nitrogen and glutathione in pig embryos at various stages of development

LENGTH OF EMBRYO	NUMBER OF EMBRYOS	AVERAGE WEIGHT PER EMBRYO	WATER	ASH		NITROGEN			GLUTATHIONE		
				Wet	Dry	Wet	Dry	Ash-free	Wet	Dry	Ash-free
mm.		grams	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
6-7	232	0.313	94.07		*	0.699	13.13	*	0.062	1.04	
10	270	0.500	93.37	0.558	8.43	0.861	12.99	14.18	0.077	1.16	1.26
15	316	0.93	91.38	0.775	9.00	1.061	12.31	13.52	0.119	1.38	1.51
30	158	2.21	91.14	0.708	8.00	1.103	12.45	13.53	0.139	1.56	1.69
50	123	6.55	91.65	1.036	12.41	0.910	10.91	12.45	0.100	1.19	1.35
60	85	14.85	91.05		*	0.966	10.80	*	0.097	1.08	*
80	41	26.00	91.59		*	0.915	10.88	*	0.092	1.09	*
100	20	72.2	91.18		*	0.95	10.78	*	0.079	0.89	*
110	22	82.2	91.02	1.30	14.50	0.972	10.82	12.65	0.078	0.89	1.047
120	15	96.2	91.26		*	0.950	10.87	*	0.078	0.86	*
160	10	238.57	91.71	1.349	16.28	0.891	10.75	12.84	0.068	0.82	0.979
200	7	488	90.34		*	1.014	10.50	*	0.061	0.63	*
240	3	725	88.7	2.58	23.09	1.233	11.01	14.29	0.052	0.46	0.59

Per cent water, 2-4 mm. embryo 97.4 per cent.

Per cent water, 2 weeks old, post-natal pig, 80.2 per cent.

* Groups in which ash was not determined.

and an aliquot taken for the iodine titration, previous tests having shown that practically all of the tripeptide was present in the reduced form.

Total sulphur was determined on the dry material by the Benedict-Denis method (Hoffman and Gortner, 1923).

Total ash was determined by placing a weighed amount of dried material in silica dishes and heating in a muffle furnace at 600°C. for 12 hours.

The experimental data. The average data on the 13 series of embryos, ranging in length from 6 to 7 mm. to 240 mm., are shown in tables 1, 2 and 3.

DISCUSSION. From the accompanying tables it is observed that the total nitrogen decreases gradually until the 50 mm. stage is reached, and

then it assumes a level which is more or less constant throughout the embryonic life. This apparent decrease in nitrogenous material is evidently more relative than actual. The high content of nitrogen at the 6 mm.

TABLE 2

Complete Van Slyke analyses of pig embryos at various stages of development together with certain other related calculations (unless otherwise noted the figures are recorded in per cent of the total nitrogen)

LENGTH OF EMBRYO	6-7 MM.	10 MM.	15 MM.	30 MM.	50 MM.	60 MM.	80 MM.	100 MM.	110 MM.	120 MM.	160 MM.	200 MM.	240 MM.
Amide nitrogen.....	7.51	7.71	7.17	7.13	7.60	7.44	7.20	7.35	7.58	7.08	6.98	7.23	7.46
Humin nitrogen.....	5.08	6.14	5.82	5.84	5.92	5.48	5.65	6.27	6.99	6.56	6.74	6.41	6.32
Basic nitrogen.....	38.04	38.79	38.54	38.57	38.35	38.11	38.58	38.61	38.72	38.62	38.19	38.28	38.15
Amino N.....	16.25	20.02	20.35	21.41	20.73	21.29	21.22	21.00	21.20	21.50	22.74	22.15	22.05
Non amino N.....	11.79	18.07	18.19	17.16	17.62	17.82	17.36	17.61	17.52	17.12	15.45	17.13	16.10
Filtrate nitrogen.....	49.97	46.87	47.51	47.83	47.02	48.23	47.60	47.70	47.53	48.25	47.64	47.51	47.38
Amino N.....	46.46	41.68	43.17	43.96	44.36	44.88	43.39	43.52	43.08	43.82	42.76	42.21	42.58
Non amino N.....	3.51	5.19	4.33	3.85	3.55	3.35	4.22	4.18	4.44	4.43	4.88	5.30	4.80
Per cent of basic N that is amino N.....	42.71	51.61	52.80	55.50	54.05	55.86	55.00	54.39	54.75	55.67	59.54	57.86	57.79
Per cent of filtrate N that is amino N.....	92.97	88.92	90.86	91.90	92.57	93.05	91.15	91.23	90.63	90.81	89.75	88.84	89.86
Arginine N, per cent.....	20.80	18.87	17.92	16.91	16.87	17.11	16.91	17.16	17.11	16.47	17.48	16.99	16.53
Cystine N, per cent.....	0.21	0.21	0.25	0.27	0.33	0.37	0.28	0.26	0.27	0.28	0.46	0.32	0.40
Histidine N, per cent.....	8.51	8.36	7.13	6.73	7.69	6.12	7.01	6.84	7.01	7.17	4.14	4.97	5.42
Lysine N, per cent.....	8.52	11.35	13.24	14.66	13.46	14.50	14.38	14.35	14.34	14.70	15.84	16.00	15.80
Per cent arginine of basic N..	54.67	48.64	46.49	43.84	43.98	44.89	43.83	44.44	44.18	42.64	45.77	44.38	43.32
Per cent histidine of basic N..	22.37	21.55	18.55	17.44	20.05	16.05	18.17	16.78	18.10	18.56	10.84	12.98	14.20
Per cent lysine of basic N.....	22.39	29.26	34.35	38.00	35.09	38.04	37.27	37.16	37.03	38.06	41.47	41.79	41.41
Per cent recovery.....	100.60	99.51	99.04	99.37	99.79	99.27	99.03	99.93	100.82	100.51	99.55	99.43	99.31

TABLE 3

Showing the per cent of total sulphur and of tyrosine in pig embryos at various stages of development

LENGTH OF EMBRYO	PER CENT OF SULPHUR			PER CENT OF TYROSINE		
	Wet	Dry	Ash-free	Wet	Dry	Ash-free
mm.						
10	0.0194	0.293	0.319	0.120	1.82	1.98
15	0.038	0.442	0.485	0.146	1.70	1.86
30	0.044	0.505	0.548	0.145	1.64	1.78
50	0.045	0.539	0.615	0.085	1.02	1.18
110	0.041	0.456	0.533	0.048	0.548	0.64
160	0.032	0.384	0.460	0.041	0.500	0.59
240	0.043	0.382	0.490	0.051	0.452	0.58

stage, which insofar as development is concerned is fairly well advanced, no doubt indicates that at the stage of implantation the products of conception are essentially pure proteins. As development continues, other substances as carbohydrates, lipins and inorganic salts, are added as con-

stituents of the embryonic framework, increasing the relative bulk of the embryo but decreasing the percentage of total nitrogen. At the point where the nitrogen becomes constant, the embryo is an adult morphologically, and any changes that may occur from this point on are changes in mass rather than any radical changes of organic structure. Apparently at 50 mm., a constant embryonic protein concentration is reached, and by so utilizing the material selectively absorbed by the placenta from the maternal circulation this level is able to be maintained with but very slight loss.

The fact that most of the decrease in nitrogen is relative is borne out by the big drop in total nitrogen between 30 and 50 mm. There is also a big rise in the ash content between these periods, and if the difference in ash is taken into consideration, the percentages of nitrogen approach one another more closely.

To a lesser extent is this decrease of nitrogen actual. A very small amount of protein material may be used for energy, but at these stages, particularly 15 to 30 mm., the placenta attachments are fairly well organized, and the embryo is no doubt receiving its sources of energy from the maternal organism.

It will be observed from the tables that amide nitrogen showed no change whatever, throughout the whole series. The humin nitrogen of the 6 mm. embryo was somewhat lower than for the later growth stages, but from the 10 mm. through to the 240 mm., the humin shows only very slight,—probably insignificant increase.

Surprisingly, the total basic nitrogen was essentially constant throughout; however, the amino and non-amino nitrogen of the bases showed varied distributions. The amino nitrogen increased as the embryonic length increased while the non-amino nitrogen necessarily decreased.

The most remarkable changes occurred in the constituents of the basic fractions. There was a decidedly definite change in the "arginine" content. The nitrogen distributed in this fraction showed a rapid decrease from the 6 mm. stage to the 15 mm. stage, then a more or less gradual decrease until the 160 mm. stage, where there was a slight rise. The most instructive change, however, occurred in the embryos of the first two groups.

Arginine has been considered by Kossel (1896) to act as the nucleus of the protein molecule, and a compilation of existing data for the arginine content of proteins has been collected by Larmour (1928) to show the relationship of the amount of basic nitrogen to the amount of arginine in the molecule, and hence the governing importance of the latter. But in our determinations we have the total bases remaining the same with a significant decrease in arginine, indicating the formation of other bases which are likewise precipitated by the phosphotungstic acid and whose

proportion of the total nitrogen is within the limits of experimental error, practically the same. It has been stated that arginine gives rise to guanidine and this is the precursor of creatin. This would not account for the rapid decrease, however, as the creatin formed would be precipitated by phosphotungstic acid and later contribute ammonia under the treatment to which the "arginine" fraction is submitted.

From the above it is adequately demonstrated that the more embryonic the tissue, the more "arginine" is present. It might be well to mention that here a strange but logical similarity exists between some facts in embryonic chemistry and tumor chemistry. A number of investigators have pointed out that the more malignant a tumor the more embryonic in nature are its cells. It also has been shown that the more malignant tumors certainly contain more arginine. The growth rate of any tissue may be considered as being dependent, at least in part, upon the amount and nature of the substances available for its nutrition. In recent studies on tumor tissue, experimental evidence has been brought forward to show that arginine definitely induces an increase in the rate of growth (Gilroy, 1930). Moreover, it has been observed that the rate of tumor growth in pregnant individuals is considerably slowed, due to the demand of the embryonic tissues for nourishment, which may ultimately be explained by considering this to be a demand for arginine. It is highly probable, therefore, that this amino acid is necessary for the nutrition of any tissue in which cell reproduction is proceeding rapidly. In both instances it may be stated that arginine is required for nuclear synthesis and cell proliferation.

The rapid decrease in the early stages seems to indicate that nuclear synthesis and cell proliferation are taking place at a greater rate than arginine is supplied to the embryo. Indeed, at that period, when the rate of fall of arginine decreases, all the organs or foundations of embryonic organs have been formed (12-15 mm.) and from then on it is only a matter of growth and slight differentiation.

Histidine also shows a definite decrease. As was expected, the largest variations occur in this group, since in the method used the errors tend to accumulate here. If one considers the early work of Ackroyd and Hopkins (1916) he undoubtedly will conclude that arginine and histidine are interconvertible in the body, and the presence of one or the other is essential for growth processes. It has been later shown, however, that they are not necessarily mutually replaceable but that histidine is far superior to arginine.

The researches of Rose and Cook (1925) indicate that histidine is necessary for nuclear synthesis so it, like arginine, decreases during the period of most rapid growth and differentiation. Histidine, along with arginine, is no doubt the chief precursor of the purine bases which, as we know, are synthesized by the embryo.

The lysine fraction showed a pronounced increase. Large variations also occur in this group. The free nature of the epsilon-amino group gives to lysine a unique importance in the protein molecule and renders it probable that this amino acid would be associated in some way or other with whatever intra-molecular change might take place. However, it must be again stated that the increase in this fraction may very well not be due to any important changes in the actual lysine content, since the material was not a pure protein.

There was no significant variation in the cystine as determined. The actual increase was very small indeed while the relative increase was comparatively large as can be seen from the tables.

The attraction of applying evolutionary succession to embryonic life suggests a new comparison. It has been recently reported (Rosedale and Morris, 1930) that there is a decrease in the amount of "histidine" nitrogen and an increase in "lysine" as we go up the animal scale. While the authors do not make the statement, their tables also show a definite decrease in "arginine." It will be recalled that this is precisely the trend of events as they occur in the developing pig embryo. The above results seem to be unique in bringing forward another bit of evidence, that the transitory changes in embryonic life seem to possess recapitulatory significance.

The nitrogen of the filtrate from the bases shows a sharp decline from 6 mm. to the 10 mm. stage and then assumes a more or less constant value. The amino nitrogen of the filtrate shows a general tendency to decrease while the non-amino nitrogen increases.

It is interesting to note the relationship of the various basic fractions to the total basic nitrogen. The "arginine" fraction, forming 54.67 per cent of the basic nitrogen at the 6 mm. embryo stage, only represents 43.32 per cent at the 240 mm. stage; the "histidine" fraction, forming 22.37 per cent of the total basic nitrogen at the 6 mm. stage, only forms 14.20 per cent at the 240 mm. stage; while, on the other hand, the "lysine" fraction, showing only 22.39 per cent at 6 mm., increases to 41.41 per cent at 240 mm.

The variations of the tripeptid, glutathione (glutamyl-cystinyl-glycine) were very striking. This substance showed a steady rise reaching a peak at about 30 mm. and then decreasing regularly again. The almost universal distribution of this oxidation-reduction system seems to indicate it must be of some vital importance to the cell. However, its content in any one tissue has not as yet been correlated with the predominance or comparative absence of any one type of metabolism and the evidence seems to be very meager for the statement that the greater the glutathione content, the greater is the rate of tissue respiration. In fact experiments have been performed which show that there is no change in reduced glutathione during normal tissue respiration.

Glutathione occurs in the cells largely in the reduced form, yet knowledge of what reduces it remains much as it was when Hopkins (1921) first announced that the disulphide form of glutathione was reduced to the sulphydril form when incubated with kidney, liver or skeletal muscles.

The part played by the tripeptid glutathione in the life of the cell is still obscure in the extreme, and it is quite possible that it is connected with protein metabolism rather than with tissue respiration. It is observed that it rises during the most rapid decrease of total nitrogen, arginine and histidine. It rises during the early embryonic life,—that active period of nuclear synthesis and cell differentiation. When things seem to have reached a more or less stabilized condition, the percentage of glutathione falls. This decline after the 30 mm. stage might be accounted for by assuming that there is at this period a protein fixation of the tripeptid. This fixation may involve glutathione as an intact molecule or its splitting up into pyrrolidone carboxylic acid and cysteinyl-glycine; the dipeptid being fixed to the protein molecule while the pyrrolidone carboxylic acid presumably is the first step in the synthesis of proline and oxyproline which may be later utilized in the formation of hemoglobin.

Total sulphur presents another interesting trend. There is a sharp increase reaching a peak at 50 mm., followed by a gradual decline.

In the 10 mm. embryo the percentage of ash is already 8.43 per cent which indicates how rapidly the embryonic tissue is accumulating inorganic materials. At the 15 mm. stage there was a slight rise, of only about one-half per cent, followed by a fall at the 30 mm. stage to approximately the original level. This fluctuation, although small, was found to be consistent in the several samples examined, and probably indicates the more rapid building up of organic molecules than inorganic materials. This decrease in ash occurs while the glutathione content is at its peak. Between 30 and 50 mm. there was a rapid deposition of ash probably due to the beginning of skeletal development, while between 50 and 240 mm. there was a gentle but regular increase.

The play of color shown in the various ashes was surprising. The 10, 15 and 30 mm. ashes were a pale blue; the 50–110 mm. ashes were white, while the 160–240 mm. ashes ranged from a faint pink to a brick red. The red color in the latter stages was presumably due to the iron oxide formed from the iron-hemoglobin complex, but the cause of the bluish tint in the younger stages remains to be determined. These colors, without a doubt, present mute evidence toward certain pronounced fundamental changes in the elementary composition of the various ashes.

The comparison of the total water content of these embryos at different lengths has proven to be quite instructive. In the very small embryo, 2–4 mm. (15 days), the water content was 97.6 per cent. There is, however, some doubt of the accuracy of this figure as representing the water

content of the embryo itself since at these stages it is impossible to separate with any degree of definiteness the extent of the embryo from that of the embryonic adnexa. The per cent of water fell rapidly from this figure to the neighborhood of 91 per cent at 15 mm., at which level it remained, fluctuating within the limits of experimental error, until the 160 mm. stage was reached. Here a second decline began and reached a figure of 88.7 per cent at 240 mm.

It is well known that mammalian embryos during the course of development have, at various times, three types of kidneys; the pronephros, the mesonephros and the metanephros. The pronephros do not function in the higher vertebrates, but it has been quite definitely proven that the mesonephros and the metanephros both are active during the brief uterine sojourn. Now at 15 mm. the mesonephros are very well developed and extend approximately one-half the entire length of the embryo. The relative size of the embryonic kidneys to the entire embryo immediately suggest their importance in fetal life. At this point, where the water content begins to assume a constant level, the mesonephros have, with all probability, begun to function as an excretory organ, controlling the water equilibrium until the 160 mm. stage is attained.

It will be remembered from embryology that the metanephric kidneys are not transformations of the mesonephric type but are of entirely separate, though like origin, springing up and developing entirely independently. In the 100-mm. pig embryo the metanephros does not yet approach one-third the size of the mesonephros, nevertheless they exist side by side, the metanephros growing and developing and the mesonephros remaining constant and finally atrophying when its services are no longer needed. At about 160 mm. the metanephric kidneys, a more discriminating type, presumably take over the excretory function. Their threshold for water is lower than that of their predecessors, and consequently there is a gradual decline in the water level to assume a new equilibrium which is not ultimately established until some time after birth.

As we know, there is a distinct loss of weight in the newborn due to loss of water. This is to be expected since, on the spur of the moment, the animal is confronted with combating three large, hitherto unknown, avenues of water escape; the exposure of a relatively large surface to air of varying degree of saturation, the acts of respiration and the loss by body excretions. We know also that there are drastic anatomical changes accompanying birth, such as the closing of the foramen ovale, the occlusion of the ductus arteriosus, the obliteration of the round ligament of the liver, to say nothing of the partial obliteration of the intra-abdominal umbilical arteries and vein. So it is quite in harmony with the existing circumstances, when we visualize such chemical drastic changes within the cells themselves, whose colloidal material must necessarily attain a new water

level in agreement with terrestrial life. This must be a general level determined on the one hand by the water intake as water itself and water acquired as a result of metabolic activities, and water output by cutaneous evaporation, respiration and excretion. This postnatal equilibrium is regulated in the main by the metanephric kidney.

Certain of the results were expressed in terms of the wet embryo, since such computations more closely approximate the conditions as they exist in the living and natural forms. The ash and the sulphur curves are of the same type as presented on the dry basis, showing similar and corresponding rises and depressions.

The results of the total nitrogen are apparently entirely different. From 6 to 30 mm. there is an increase in the percentage of total nitrogen instead of a decrease as shown on the dry basis. Between 30 and 50 mm. there is a slight fall to a constant level which is maintained to the 160 mm. stage, after which there is a continued increase.

In comparing the two series of nitrogen results it appears that while both water and nitrogen (dry basis) show an initial decrease, the decrease in water is so much greater than is that of nitrogen that when calculations are made on the wet basis the curves assume a positive slope. The nitrogen calculated on the dry basis is constant from the 50 mm. stage through the 240 mm. stage. On the wet basis the constancy only persists through the 160 mm. stage, after which there is a slight rise. This rise, however, begins where the total water content begins its second continued decline, and therefore is apparently relative rather than actual.

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SUMMARY

The preceding results were obtained from a detailed study of 1552 pig embryos varying in length from 2-4 mm. to 240 mm. It was observed that:

1. The total nitrogen decreased gradually until the 50 mm. stage was reached and then assumed a level which is more or less constant throughout embryonic life.

2. Amide nitrogen, humin nitrogen and cystine nitrogen showed no significant changes.

3. The total nitrogen of the bases was constant throughout. The basic

amino nitrogen, however, increased while the basic non-amino nitrogen necessarily decreased.

4. The most remarkable changes occurred in the constituents of the bases. There was a decrease in "arginine," a decrease in "histidine" and an increase in "lysine." Most of these changes took place prior to the 30 mm. stage and were quite definite.

5. The nitrogen of the filtrate from the "bases" showed a decrease. Tyrosine, determined separately, also showed a distinct decrease as embryonic life progressed.

6. Glutathione increased rapidly in the early stages reaching a peak at 30 mm. and then declining. It was suggested that glutathione possibly aids in protein synthesis and in the production of proline and oxyproline, which later may contribute to the formation of the hematin molecule.

7. Total sulphur showed a sharp increase to the 50 mm. stage after which there was a gradual decline.

8. The ash content presented an initial increase between the 10 and 15 mm. stages, which was followed by a slight but consistent decrease to the 30 mm. stage. There was a rapid increase in ash from the 30 mm. to the 50 mm. stage and a constant but more gradual increase from this latter stage to the 240 mm. embryo.

9. Total water follows a rapid decrease from the 4 mm. to the 15 mm. embryo after which there is a remarkable constancy which is maintained until the 160 mm. stage is reached. From this latter stage there is a gradual decline in the water content until birth, the final water equilibrium only being reached at an early period in postnatal life.

The idea is advanced that the embryonic equilibrium is maintained by the mesonephros and the second decline only occurs when the metanephros assumes the excretory function.

10. The recapitulatory significance of the variation of the bases was pointed out, "arginine" and "histidine" decreasing while "lysine" increases. This is exactly as the distributions vary as we go up the animal evolutionary scale.

11. The relationship between embryonic chemistry and tumor chemistry was also indicated. The youngest embryos have the highest "arginine" content. This is quite significant, particularly when one correlates it with the fact that the more embryonic a tumor tissue the more "arginine" is found in its contents.

12. And finally, this investigation has brought out the fact that in spite of the constant indirect communication with the maternal organism, mammalian embryos follow certain definite and fixed chemical courses during development which appear to be governed by the inherent nature of the embryo itself and the specific selective adsorption of the placenta rather than any variations in the maternal nutrition, and, from the stand-

point of regularity and gradation of changes mammalian embryos can be studied equally as well as embryos representing comparatively closed systems, with an added advantage of presenting chemical variations distributed over a longer prenatal period.

BIBLIOGRAPHY

- ACKROYD, H. AND F. G. HOPKINS. 1916. *Biochem. Journ.*, x, 551.
- BUGLIA, G. AND A. COSTANTINO. 1912a. *Zeitschr. physiol. Chem.*, lxxxi, 143.
- 1912b. *Zeitschr. physiol. Chem.*, lxxxi, 155.
- CALVERY, H. O. 1929a. *Jour. Biol. Chem.*, lxxxiii, 231.
- 1929b. *Journ. Biol. Chem.*, lxxxiii, 649.
- CAVETT, J. W. 1932. *Journ. Biol. Chem.*, xcv, 335.
- FOLIN, O. AND A. D. MARENZI. 1929. *Journ. Biol. Chem.*, lxxxiii, 89.
- GILROY, E. 1930a. *Biochem. Journ.*, xxiv, 589.
- 1930b. *Biochem. Journ.*, xxiv, 1659.
- GORTNER, R. A. 1913. *Journ. Amer. Chem. Soc.*, xxxv, 632.
1914. *Journ. Amer. Chem. Soc.*, xxxvi, 1556.
- GORTNER, R. A. AND M. J. BLISH. 1915. *Journ. Amer. Chem. Soc.*, xxxvii, 1630.
- HAUGE, S. M. 1931. *Journ. Amer. Chem. Soc.*, liii, 3049.
- HOFFMAN, W. F. AND R. A. GORTNER. 1923. *Journ. Amer. Chem. Soc.*, xlv, 1033.
- HOPKINS, F. G. 1921. *Biochem. Journ.*, xv, 286.
- KOSSEL, A. 1896. *Zeitschr. physiol. Chem.*, xxii, 176.
- LARMOUR, R. K. 1928. *Trans. Roy. Soc. Canada*, xxii, 349.
- NEEDHAM, J. 1931. *Chemical embryology* (3 vols.). Cambridge Univ. Press.
- PLIMMER, R. H. A. AND J. L. ROSEDALE. 1925. *Biochem. Journ.*, xix, 1004.
- ROSE, W. C. AND K. G. COOK. 1925. *Journ. Biol. Chem.*, lxiv, 325.
- ROSEDALE, J. L. AND J. P. MORRIS. 1930. *Biochem. Journ.*, xxiv, 1294.
- TUNNICLIFFE, H. E. 1925. *Biochem. Journ.*, xix, 194.
- VAN SLYKE, D. D. 1911. *Journ. Biol. Chem.*, x, 15.

STUDIES OF THE INFLUENCE OF PULMONARY MOTION AND DISTENTION ON MOVEMENTS OF THE THORAX

I. CHEMICAL AND NERVOUS FACTORS INVOLVED IN ESTABLISHING APNEA

II. THE RELATION BETWEEN PULMONARY AND THORACIC MOVEMENTS

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PART I. CHEMICAL AND NERVOUS FACTORS INVOLVED IN ESTABLISHING APNEA. Pulmonary ventilation is adapted to the varying needs of the organism by several different regulatory mechanisms. The factors acting on the respiratory center through the circulating blood, so-called chemical regulation, have been studied by physiologists and clinicians during the last decade, while the problems of nervous regulation have been somewhat neglected.

Physiologists, and in particular clinicians, until recently have been satisfied with the Hering-Breuer (1) theory of auto-regulation. According to this conception, the respiratory movements are regulated through the vagus nerves, each distention of the lungs occasioning a centripetal impulse resulting in an expiration, and each collapse a centripetal one resulting in an inspiration. In the physiological literature several experimental observations have appeared which are, however, opposed to this theory and which suggest a different and more complicated nervous control.

Neither chemical nor nervous factors alone regulate respiration; for, as Haldane (2) states, "We cannot separate the nervous from the chemical control of breathing, since each determines the other at every point." It is, therefore, only in combination with chemical factors that the nervous influences can be adequately investigated. For this study we have selected a relatively simple problem, so-called vagal apnea.

It has been frequently demonstrated (Lockenberg (3), Guttermann (4), Head (5), Christiansen and Haldane (6)) 1, that a period of apnea following over-ventilation is prolonged by distention and terminated or shortened by collapse of the lungs; 2, that during normal ventilation, pulmonary distention produces a period of apnea; and 3, that distention of the lungs retards the respiratory rate. These phenomena do not occur after section of both vagi. Because distention of the lungs with nitrogen results in a period of

apnea, just as when the lungs are distended with air, it has been thought that a purely nervous mechanism is implicated. This conception was held at a time, however, when oxygen was still considered to be the most important gas in the mechanism of respiration. Haldane and his collaborators have demonstrated that apnea produced by distention can be prolonged by previous over-ventilation, and shortened by administration of carbon dioxide, and have shown, therefore, that so-called vagal apnea can be influenced by chemical factors. They hold the view that this is not true apnea, but markedly prolonged expiration, and "that the term vagus apnea is an entire misnomer."

In order to learn how great an influence each of these factors, chemical and nervous, has on apnea resulting from distention, it was necessary to choose a definite and readily manifest point or level in chemical regulation. This level is probably represented by the threshold value of the respiratory center, which we define as the level to which the strength of the stimulus in the blood must rise during apnea induced by over-ventilation, before a respiratory movement occurs. The use of this level for this purpose involves the same principle used by Filehne (7) and Knoll (8), and later by Wieland (9), to ascertain the degree of irritability of the respiratory center.

Since the threshold stimulus is not necessarily identical with the usual respiratory stimulus, it was necessary to learn if, and how far, the threshold value of the respiratory center is influenced by pulmonary distention and collapse. To this end a series of samples of blood was taken for analysis during the latter part of, and at the end of, a period of artificially induced apnea.

EXPERIMENTAL PROCEDURE. The following experimental procedure was employed. Only dogs were used. The animals were anesthetized with chloralose injected intravenously in a dosage of 0.1 gram per kilogram of body weight, supplemented occasionally with small amounts of the drug if they became restless. A tracheal tube was inserted. The natural minute volume of respired air was measured. Later, artificial respiration was maintained by means of a Starling pump, the stroke volume and rate of which were so adjusted as to give approximately the desired respiratory minute volume. Bilateral pneumothorax was then created. At first this was accomplished by removing at least 6 cm. of two middle ribs on each side. Later, the sternum was split from above downward to the point at which the 6th or 7th costal cartilage was attached. Incisions from the lower end of this opening were then made by cutting laterally along the borders of the 7th ribs. The flaps so made were fastened back. When thoracic movements occurred, the lower intact portion of the thorax was found to move freely and independently of the motion of the lungs. A Y-shaped glass cannula was so introduced into a lateral branch of the deep femoral

artery that the opening lay almost in the main artery. By this device the flow of arterial blood past the mouth of the cannula was free and uninterrupted. Heparin was then added to the dog's blood and the cannula closed with rubber tubes and clamps.

The ventilation was varied by appropriate adjustments in the stroke and rate of the Starling pump in order to bring about both over- and under-ventilation. Distention of the lungs was effected in one of two ways: 1, when sudden distention during a period of apnea was desired, room air was introduced into the lungs of the animal from an air-tight 3-liter glass jar by suddenly distending a rubber bag contained within it; 2, if distention was to be maintained during ventilation, expiratory resistance was increased by submerging the expiratory tube in water to a desired depth. A three-way stopcock inserted into the inspiratory tube permitted collapse of the lungs or the omission of ventilation during one or more strokes of the pump.

The records were made on smoked paper by writing levers. Intra-tracheal pressure was recorded by a Marey capsule connected by a side opening with the intra-tracheal tube. Abdominal respiration was recorded by transmitting changes of pressure in a rubber bag held snugly to the abdomen by a leather cuff to the lever of a Marey capsule. The movements of the lower thorax were transmitted by thread over pulleys to a rod supporting a recording lever and moving in a vertical bearing. The arrival of the piston of the pump making and breaking contact at the end of the expiratory stroke was recorded by a signal magnet.

A number of samples of blood were drawn in rapid succession from the femoral cannula and deposited in test tubes under liquid paraffin by means of a special rotating stopcock.¹ Carbon dioxide and oxygen of the blood were analyzed according to the method of Van Slyke and Neill (10). The pH of the blood was measured by the colorimetric method of Hastings and Sendroy (11).

The volume of air supplied by the pump was regarded as enough to bring about either over- or under-ventilation depending upon whether it was greater or less than the volume breathed spontaneously by each dog after the introduction of the tracheal cannula. Over- or under-ventilation having been attained, artificial ventilation was stopped. The lungs were then either allowed to collapse by removing the expiratory resistance, or were

¹ This stopcock was made of glass and first used in January, 1931. Blood entered through an opening in the bottom of the center plug emerging on the side. Eight perforations through the jacket in a circle at the level of the opening in the center plug lead, by rubber connections, to openings into the lower ends of the glass sample tubes. The sample tubes are held by a stout rubber band against the outer surface of the jacket. The whole apparatus is then filled with paraffin oil. By revolving the jacket with the sample tubes fixed to it, the tubes can be successively and rapidly connected to the arterial needle or cannula.

distended with room air by the method just described. A series of samples of blood was taken toward the end of the period of apnea. To avoid differences in the general state of the animals, the time between the taking of the samples was as short as possible (15 minutes). Because over- and under-ventilation are responsible for marked disturbances in the acid base equilibrium, the periods of ventilation preceding the taking of samples were equal in duration.

The question at once arises: is the sample of blood taken from the femoral artery identical in composition with that arriving at the respiratory center at the same moment? Identity would, it seems, depend on whether the two points are equidistant from the heart, and on whether the velocity in the two arteries is the same. The distance to the femoral artery is obviously greater than that to the respiratory center, but the difference may be compensated in whole or in part by decreased velocity in the pre-capillary vessels supplying the center. The discrepancy in time cannot be more than 3 or 4 seconds and need not be considered in this connection since the same difference exists both in the state of distention and in that of collapse.

RESULTS. The direct influences of distention and collapse of the lungs on the respiration have already been described. The term distention, or degree of distention, is used to designate only the static factor; that is to say, the state of being distended in distinction to the state of being collapsed. The use of the term is intended to exclude any dynamic factors as far as the lungs are concerned.

When artificial ventilation was stopped with the lungs in *collapse*, thoracic movements began in 14 seconds, with a frequency of 30 per minute (fig. 1a). As soon as artificial respiration was again instituted, the depth and frequency of the thoracic movements decreased. But when artificial respiration was stopped with the lungs *distended* (fig. 1b), apnea lasted 88 seconds and thoracic movements began with a frequency of only 12 per minute. If an animal was slightly *under-ventilated*, it exhibited regular thoracic movements of its own. In collapse of the lungs (fig. 2a) no apnea resulted; the frequency was 24 per minute. In distention (fig. 2b) there was apnea for 15 seconds; thoracic movements then began with a frequency of 10 per minute. After bilateral vagotomy, collapse and distention of the lungs had no influence on the type of thoracic motion (fig. 3). That the dog under description was over-ventilated is shown by the occurrence of apnea before vagotomy; after vagotomy, this phenomenon did not recur. These experiments establish the fact that distention can occasion apnea, or prolong it, if it exists already, and can likewise decrease the frequency of thoracic motion, but only if the vagi are intact. It is also a fact that the length of an apneic period, occasioned by distention of the lungs, can be influenced by the degree of previous ventilation.

The results of the chemical analyses of the blood in all of the experiments show changes in the same direction. Three examples, therefore, suffice.

At the beginning of a period of apnea due to over-ventilation, the oxygen saturation is nearly normal, while the carbon dioxide content is at the lower limit of normal (fig. 4). If ventilation is stopped when the lungs are

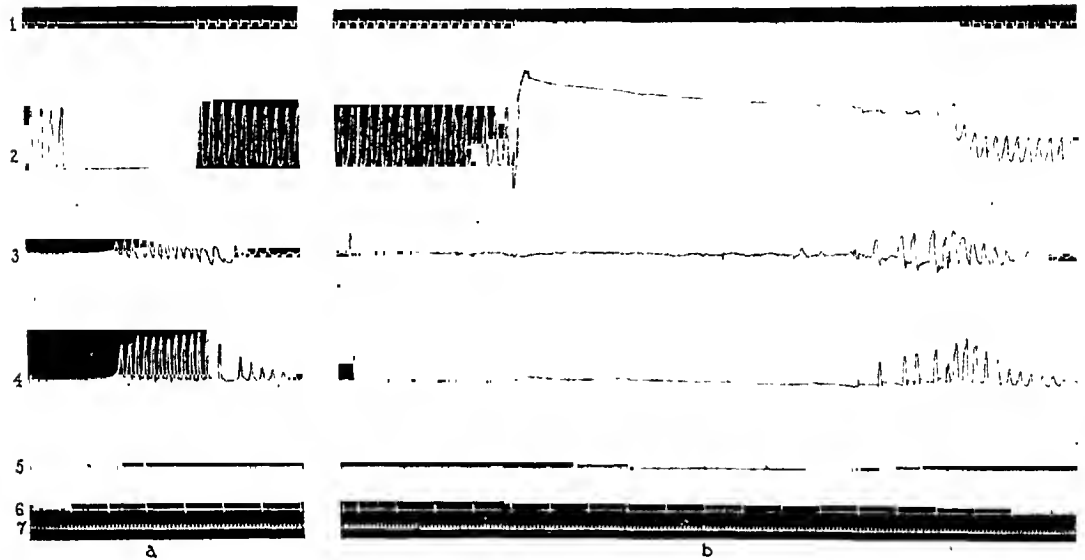


Fig. 1. The effect is shown upon the duration of apnea in a dog which has been the subject of *over-ventilation* when ventilation was arrested *a*, in the state of collapse, and *b*, in a state of distention of the lungs. The curves from above downward are made by:

1. A signal magnet in circuit with the respiratory pump. At the end of expiration, the shaft of the piston made contact.
2. A tambour connected by tubing with the tracheal cannula. Upstrokes of the curve indicate inspiration.
3. A lever moving vertically, connected by string with the lower portion of the thorax. Upstrokes represent inspiration.
4. A tambour connected by tubing with inflated rubber bags applied to the abdomen, to record motions of the abdominal wall. Upstrokes represent inspiration.
5. A signal magnet, to record the time when maneuvers were carried out; as when samples of blood were taken.
6. A time signal every 10 seconds.
7. A time signal every 1 second.

The curves in figures 2, 3, 8, 9, 10, 11 record similar events.

in collapse, the concentration of oxygen decreases and that of carbon dioxide increases, rapidly; when they are distended these changes go on much more slowly. When the lungs are in collapse the first thoracic movement takes place at a much higher oxygen, and much lower carbon dioxide concentration than when they are distended.

Although the concentration of carbon dioxide in the blood of the dog now being described was low, an indication of over-ventilation, apnea did not occur until the lungs were distended during the second part of the experiment (fig. 5). This result might have been due to low concentration of oxygen due in turn to changes in the lungs, the result probably of beginning pulmonary edema. Although pathological conditions were present in this experiment, the various changes in concentration of the gases in the

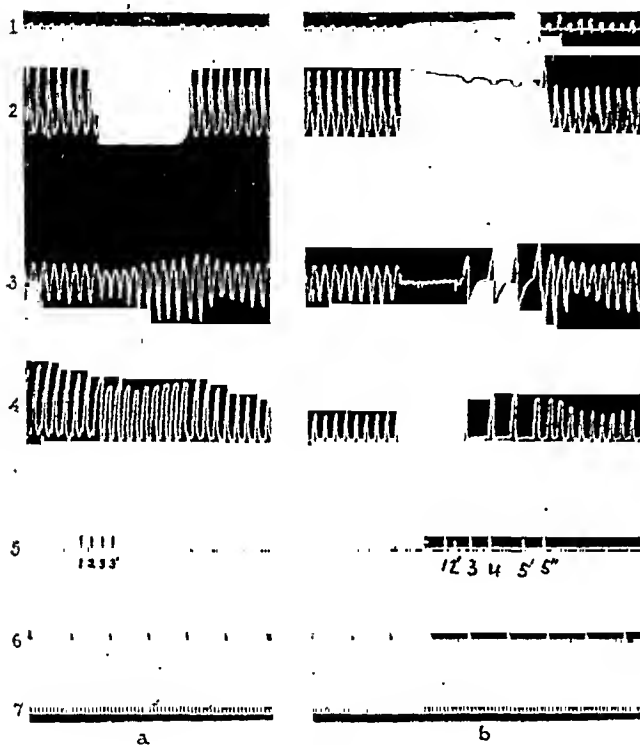


Fig. 2

Fig. 2. The effect is shown upon the duration of apnea in a dog which has been the subject of *under-ventilation* both when ventilation was arrested *a*, in a state of collapse, and *b*, in a state of distention of the lungs. The curves record events similar to those in figure 1.

Fig. 3. The effect is shown of bilateral vagotomy in a dog which has been the subject of *over-ventilation*, when ventilation was arrested, the lungs being in a state of collapse (portion of the curve to the left of the ordinate) and in a state of distention. The curves represent events similar to those in figure 1.

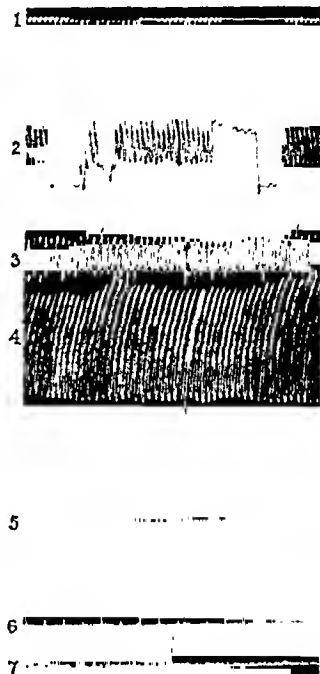


Fig. 3

blood at the onset of the animal's thoracic movements, after collapse and after distention of the lungs, were in the same direction as in the first experiment described. Since a period of apnea did not occur during collapse, the threshold value was not obtained. The recorded value for the level of the stimulus of the blood was, of course, greater than the true

threshold value. The difference, therefore, between the threshold stimulus in distention and collapse is really greater than that shown.

Somewhat different results are exhibited in figure 6, obtained from an animal in which the saturation of oxygen at the beginning of the apneic

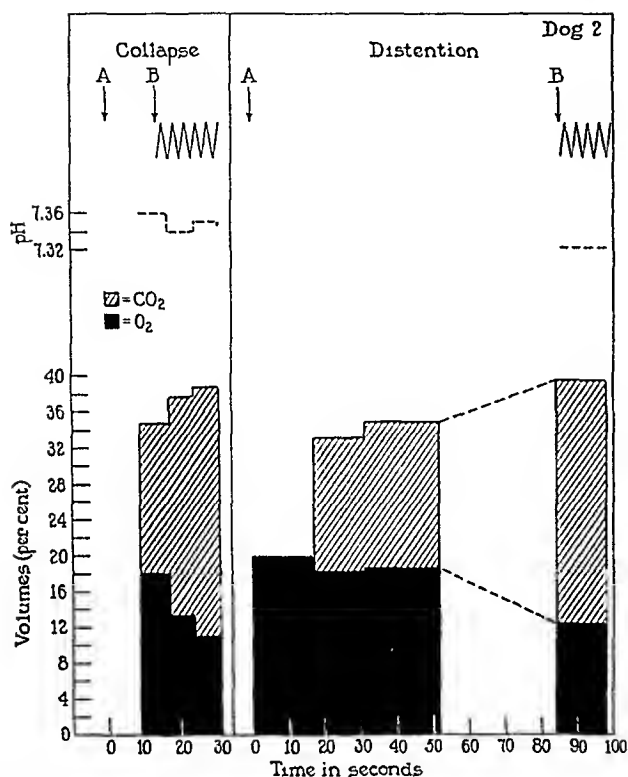


Fig. 4

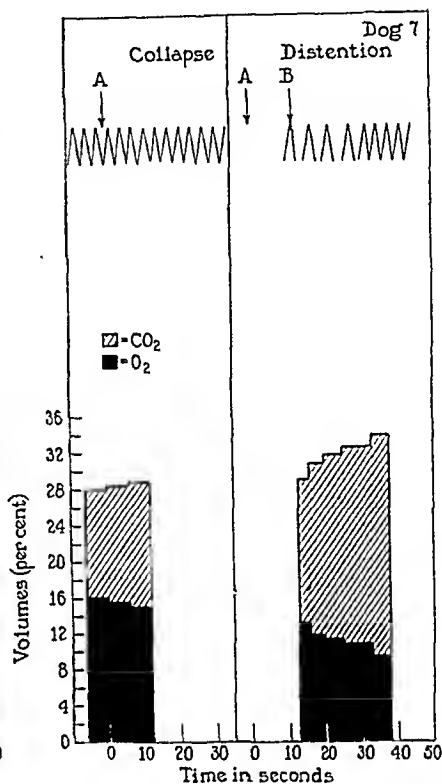


Fig. 5

Fig. 4. Chart of the relation of the concentrations of carbon dioxide and oxygen in the blood of a dog previously over-ventilated by artificial means to the onset of thoracic movements following discontinuance of artificial ventilation with 1, collapse, and 2, distention of the lungs. Dog 2, the same one from which the tracings in figure 1 are taken is the subject of this chart. In this figure and in figures 5 and 6 the arrows at *a* indicate the moment at which artificial ventilation was stopped, those at *b* the moment at which thoracic movements began. The line immediately beneath the arrows shows the periods during which thoracic movements occurred. Apnea was otherwise present. Hydrogen ion concentration of the blood is shown in broken lines.

Fig. 5. Chart showing the same relations as those described in figure 4, but with the dog under- instead of over-ventilated. The tracings in figure 2 are taken from the same animal (dog 7.) It is to be noted that no apnea occurred when the lungs were collapsed.

period was nearly normal. Since there had been over-ventilation, the content of carbon dioxide was low. While the period of apnea lasted only

3 seconds when the lungs were in a state of collapse, the duration when distended was 77 seconds. It is apparent from these three experiments that the changes in the gases of the blood progress more slowly when the lungs are distended than when they are in collapse.

Before passing on to a discussion of these results it is to be noted that in all cases the apnea which occurred during distention was true apnea and not merely prolonged expiration as described by Haldane.

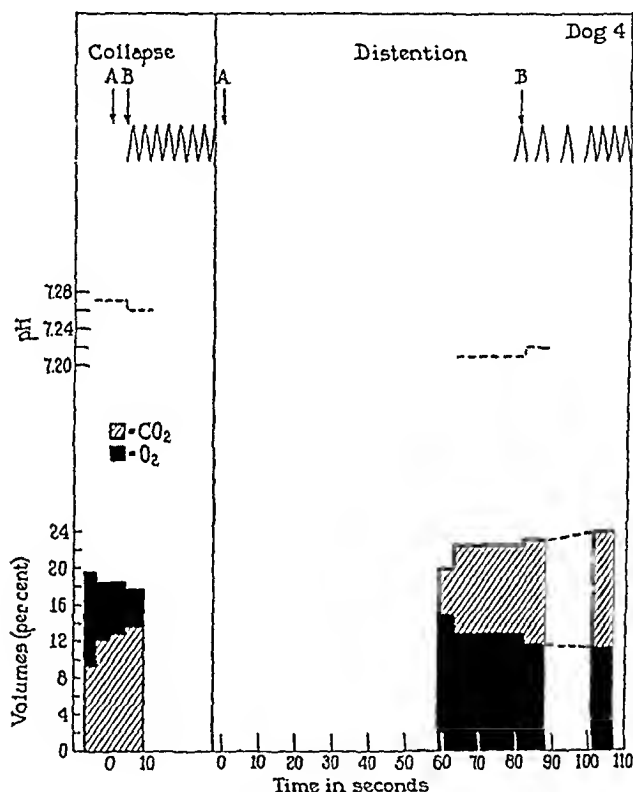


Fig. 6. Chart showing the same relations as described in figure 4 in a markedly over-ventilated animal.

DISCUSSION. If the first thoracic movement marking the end of apnea denotes the moment at which the strength of the stimulus in the blood reaches the threshold value required to actuate the respiratory center, it is apparent, as the result of these experiments, that this value is greater in distention than in collapse of the lungs. The mechanism responsible for this difference may, however, be very complicated. For instance, the medullary centers for the thoracic muscles may change their sensitivity to the impulses coming from the respiratory center. We can only say, therefore, that distention of the lungs has the same effect as decrease in irritability of the respiratory center. Furthermore, following apnea when the

lungs were in collapse, thoracic movements began at a much more rapid rate than when they were distended, which fact strongly suggests that the degree of distention of the lungs has an influence also on the character of thoracic motion.

We can confirm the fact that after bilateral vagotomy, distention of the lungs does not occasion apnea, nor does distention or collapse any longer influence the rate of the thoracic movements. From vagotomy experiments such as these, many authors have concluded that the centripetal impulses produced by distention and by collapse of the lungs run in the vagus. This conclusion is not justified as long as other nervous communications exist (as in the Plexus vagi).

The appraisal of this form of experiment is difficult and requires great caution because the removal of vagal influence changes the sensitivity of the respiratory center to other stimuli such as that of carbon dioxide. It might, for instance, become less sensitive to impulses coming to it through other nervous pathways. This possibility has already been discussed by Breuer. He says "One could suppose that the peripheral stimulus produced by lung distention acts on the medulla as before, and therefore does not go by way of the vagus; but the respiratory center has become too insensitive, too insufficiently labile, for stimuli to produce marked changes in respiratory rhythm." Breuer thinks that this theory is unlikely, although no experiments have been performed which exclude the possibility of such a mechanism. It is therefore necessary for the present to conclude no more than that the degree of distention of the lungs has an effect on respiration only when the vagi are intact.

There remains for discussion the question as to how the centripetal impulses due to distention of the lungs arise. That intra-thoracic pressure is of no importance, as Breuer has already shown, becomes apparent from the fact that no changes occurred in our experiments since the thoracic cavity was in open communication with the atmosphere. It is more difficult to decide whether distention of the pulmonary tissue itself or change in intra-alveolar pressure acts as the stimulus, since these two, being interdependent to a marked degree, can probably not be separated. Since only the difference between intra- and extra-alveolar pressure can be effective, to test the influence of respiration under negative pressure is of no avail. The possibility that centripetal impulses result primarily through the influence of distention of the lungs on the extra-pulmonary circulatory apparatus, complicates the problem; the reference to this possibility is due to the fact that in recent investigations it has been learned that changes in blood pressure influence the respiration (12), (13). Heymans has shown that a rise in blood pressure in the carotid sinus may be accompanied by the occurrence of apnea. In the course of these experiments marked dilatation of the right ventricle, suggesting impairment of the circulation, was ob-

served during a period of over-distention of the lungs. Under this condition the systemic blood pressure and, therefore, the pressure in the carotid sinus seemed to have decreased and yet decreased pressure renders the participation of this mechanism in apnea during distention unlikely. Further investigation of this problem is necessary.

If the degree of distention of the lungs exerts important influences on the behavior of the respiratory center, it becomes necessary to keep the volume of the lungs constant in experiments dealing with the regulation of respiration. How sensitive this aspect of the whole mechanism may be is suggested by the fact that functional residual air (Binger) (normal capacity, Rohrer), a factor in establishing the degree of distention, varies with position. It is greater in the upright than in the prone position and this may be sufficient to change the irritability of the respiratory center, and hence the character of respiration.

SUMMARY

1. In dogs, when the chest is laid open, the influence of distention and collapse of the lungs on the duration of the period of apnea has been studied. By taking successive small samples of arterial blood toward the end of the period, the threshold value of the respiratory center was ascertained.

2. Distention of the lungs is responsible for the onset of apnea or prolongs this state if it exists already, and slows the respiratory (thoracic) rhythm.

3. In distention, respiratory movements do not occur until the oxygen concentration in the blood is lower and the carbon dioxide higher than in collapse.

4. Distention of the lungs may be said, therefore, to influence respiration in the sense of decreasing the irritability of the respiratory center.

5. The influence of pulmonary distention disappears after bilateral vagotomy.

PART II. THE RELATION BETWEEN PULMONARY AND THORACIC MOVEMENTS. Traube (14) has pointed out that distention of the lungs is followed on each occasion by an expiratory movement, and collapse of the lungs by an inspiratory one. This observation has been confirmed several times. Breuer also found that this relation exists provided the individual insufflations are large enough to create appreciable differences in the volume of the lungs. If, however, the insufflations are so small and rapid that no significant change in volume occurs, the thoracic movements are entirely independent of the rhythm of insufflation. This complex or phenomenon was generally regarded as evidence of the auto-regulatory mechanism described by Hering and Breuer. In the literature, and in our own experiments, we find that both in distention and in collapse, regular thoracic movements may occur without any change in pulmonary volume, an

occurrence which is contrary to the behavior one would expect if the Hering-Breuer theory were wholly correct. This study was begun, therefore, in a search for other regulatory mechanisms.

In the preceding part of this paper it was shown that distention of the lungs decreased the sensitivity of the respiratory center, and changed the respiratory rhythm. We wished, therefore, to investigate the extent of the influence of distention of the lungs on the relation between their movement and the motion of the thorax. Observations were first made on the relation of spontaneous movements of the thorax to movements forced

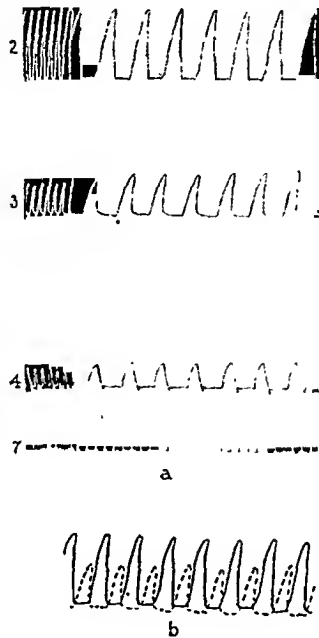


Fig. 7. The time relations are shown during artificial ventilation of a dog. The curves record events similar to those described in figure 1 except that curves 1, 5 and 6 are omitted. In (a) the original curves are reproduced; in (b) a redrawing of curves 1 and 2 in their proper relations.

upon the lungs by varied conditions of artificial ventilation. The general operative procedure was similar to that described in part I.

RESULTS. If the volume of the ventilation is constant, but if the dog is kept *slightly* under-ventilated, the number of movements of the thorax depends on the number of movements of the lungs. Under these circumstances it is easy to demonstrate the relation described by Traube (15) (fig. 7). The motions of the thorax take place in the intervals between insufflations of the lungs. In the experiments if, for example, the ratio of movements of lungs to movements of thorax was, under a certain set of

conditions, 1 to 1, this ratio changed when the number of insufflations, having been 16 per minute, was decreased to 11; there were then two thoracic movements instead of one (fig. 8). When the insufflations were stopped (incident to manipulation of the pump) the rate of the thoracic movements increased. If the rate of insufflations was raised to 16 per minute the 1 to 1 rhythm was resumed at once. A reduction of the rate to 9 again occasioned a 2 to 1 rhythm. Following this slow rate of movement of the lungs, there were found during the periods of collapse, equal and regular thoracic movements (fig. 8).

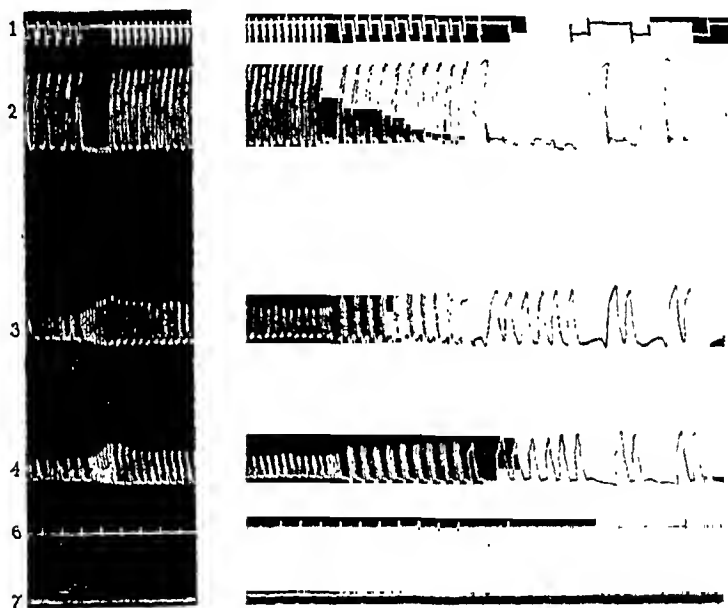


Fig. 8. The effect is shown of changes in frequency of ventilation (strokes of the pump) on the thoracic and abdominal motions of a dog. The division of the curve into two parts indicates an interval of $2\frac{1}{2}$ minutes. At first, reading from left to right, for each stroke of the pump there are two of the thorax. When the strokes of the pump are increased in frequency the ratio of pump to thorax is 1 to 1 preceded and succeeded by short periods when artificial ventilation ceased and a few rapid spontaneous thoracic motions are observed. When the rate of the pump is again reduced, the ratio returns to 1 to 2. Arrest of the pump occasioned once more rapid thoracic motions. The curves record events similar to those described in figure 1 except that the signal magnet for recording the time of maneuvers is omitted.

Following section of the vagi these relations are at once destroyed. If at first a 2 to 1 rhythm is present, this changes immediately after bilateral vagotomy to an unrelated rhythm of about 9 thoracic movements to 6 distentions of the lungs (fig. 9).

DISCUSSION. Our experiments confirm the observation that regular thoracic movements may occur without changes in the volume of the lungs. They also confirm the fact that during each period of insufflation no

thoracic movements need take place. Insufflation acts apparently as an inhibitor of the respiratory center. Attempts to analyze the relations as they are exhibited in our curves, in the light of the Hering-Breuer theory, meet with failure. On examination of the curves one finds that the beginning of distention of the lungs causes an expiratory movement of the thorax, and that an inspiratory motion occurs either at the point of maximum distention or on the arrival of the lungs at the position of collapse. One difficulty is in our curves; artificial expiration is so nearly instantaneous because of the low expiratory resistance, that comparison during this phase is impossible. That the maximum distention should cause an inspiratory movement would be directly opposed to the Hering-Breuer theory, and that return to the position of collapse should likewise stimulate inspiration is unlikely, since the same degree of distention (collapse) would then cause both inspiration and expiration. It seems unnecessary to continue this discussion; it is desirable, however, to emphasize the observation that thoracic movements cease during the active phase of artificial ventilation and take place only during the intervals between insufflations.

So far as the relation of the function of the vagus nerves to these phenomena is concerned, one can conclude no more than that inhibition of thoracic movements during each period of artificial distention of the lungs occurs only when the vagi are intact as vagotomy experiments show.

Since distention of the lungs has been shown to decrease the irritability of the respiratory center, the question arises as to how far, and by what means, the degree of distention controls the relation between movements of the lungs and the thorax. A given concentration of oxygen and carbon dioxide in the blood can either inhibit or give rise to regular thoracic motion depending on whether the lungs are distended or in a state of collapse respectively. Experiments were, therefore, carried out in order to show how, without change in respiratory minute volume, alteration of the volume of the lungs affects the thoracic movements.

If the animal was *markedly* under-ventilated, increase in the volume of the lungs sometimes resulted in bringing about decrease in extent of thoracic movements, but their frequency and their temporal relation to pulmonary movements remained unchanged. Sometimes the changes in diaphragmatic tone described by Head and Hess (16) could be observed. If, however, the animal was so *slightly* under-ventilated as just to avoid apnea, moderate distention produced marked slowing of respiratory movements while considerable distention (fig. 10)² produced apnea.

² This animal, now being described, under the usual conditions of ventilation moved his thorax only during every second or third instead of during every pause between insufflations. Although the numerical relation between distention of the lungs and thoracic movements was somewhat different, the underlying principle of coördination remained undisturbed, since thoracic movements took place only during the intervals between distentions of the lungs.

With fixed respiratory minute volumes, the effect of increasing or decreasing distention of the lungs on the thoracic movements is dependent in part on the magnitude of the minute volume. The observation that slightly under-ventilated animals become apneic with increased distention of the lungs, may be explained as due to consequent diminished irritability of the respiratory center. If there were a doubt concerning the meaning of these experiments, it would arise from the fact that the blood exhibits

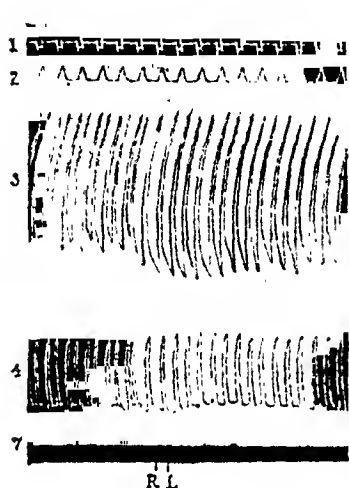


Fig. 9

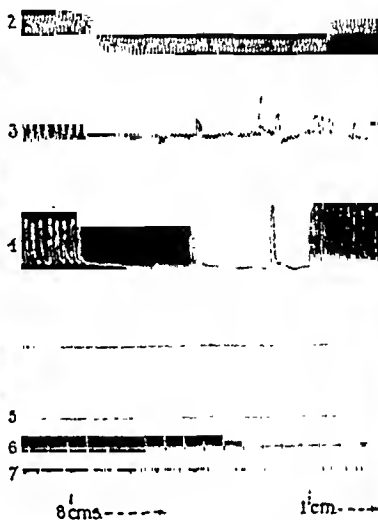


Fig. 10



Fig. 11

Fig. 9. The effect is shown on pulmonary and thoracic motions of cutting the vagus nerves. The curves record events similar to those in figure 1, except that the signal magnet to record maneuvers and the ten-second signal are omitted.

Fig. 10. The effect is shown 1, of increasing the resistance to expiration to 8.0 cm. water, and 2, of decreasing to 1.0 cm. water. The curves represent events similar to those in figure 1, except that curve 1 representing strokes of the respiration pump is here omitted and that, in the topmost curve in this figure (curve 2) inspiration is a downstroke.

Fig. 11. The effect is shown on pulmonary and thoracic motions of reducing suddenly the resistance to expiration, and so the degree of pulmonary distention, from 10.0 cm. to 0.0 cm. water. The curves represent events similar to those in figure 1 except that the signal for the respiration pump is omitted. In the topmost curve (curve 2) inspiration is an upstroke as in figure 1.

slightly greater oxygen and slightly lower carbon dioxide concentrations during periods of distention of the lungs than during control periods, the respiratory minute volume remaining the same. Because apnea occurs at once, however, it seems unlikely that change in the blood is the decisive factor in bringing about the period of apnea.

Finally, experiments were designed to show whether thoracic movements depended on the extent of the movements of the lungs. Animals, prepared as usual, were much under-ventilated. By means of markedly increased artificial expiratory resistance, great increase in the volume of the lungs was obtained.³ The expiratory resistance was then so greatly reduced, the respiratory minute volume being kept constant, that the maximum volume of the lungs in inspiration was smaller than the minimum volume had been during expiration (fig. 11). Since, when the lungs were distended, the expiratory phase failed to inhibit the respiratory center, it seemed likely that when they were smaller in full inspiration than they had been before during expiration, the degree of their distention would fail to influence the respiratory center and so bring about dissociation between motions of the lungs and motions of the thorax. But this result did not occur. The motions of the lungs and thorax remained associated, even as they had been before (fig. 11).

This experiment is valuable because although the efficiency of the same respiratory minute volume would be slightly lowered by decrease in volume of the lungs, resulting in a tendency toward increase in acidity of the blood, a tendency which would be more favorable for dissociation of pulmonary and thoracic movements, dissociation did not occur. Since these movements continued to be associated it appears that the links in the chain of causation—degree of distention of the lungs, degree of irritability of the respiratory center, inhibition of respiratory movements—are interdependent to a moderate degree only. The static factor, the degree of distention of the lungs, therefore, is not the occasion for sending relevant centripetal impulses to the respiratory center. One can conclude, in consequence, that impulses dependent upon *movement* itself of the lungs, are concerned in this association. Which mechanism, precisely, movement of the lungs sets into operation, whether it is stretching of the tissue itself, the influence of the air stream on the bronchial mucous membrane or even periodic changes in pulmonary blood pressure, is unknown. The stronger of these unrecognized influences was called into play in some of our earlier experiments (fig. 1b). After the acidity of the blood had risen during apnea above a point where it was certain to stimulate the respiratory center, it was effective in initiating motion even though the degree of distention of the lungs was great enough, under other circumstances to inhibit thoracic movements altogether. Associated motions began again immediately when the lungs were set in motion artificially (though the degree of their distention was still great). No conclusion more searching can be

³ From the curve recording intratracheal pressure, inferences regarding the volume of the lungs can be made directly. Following over-distention, a return to a smaller size would mean that the initial elasticity had been recovered. Under these conditions the volume for identical pressures would be less.

drawn than that these influences, the influence of acidity of the blood and that of motion of the lungs, are more profound than centripetal impulses arising from the degree of distention. That this inference is just is fortified by experiences with animals in which, though so markedly under-ventilated that distention of the lungs was executed without exhibiting its usual inhibitory influence, complete association persisted nevertheless.

CONCLUSIONS

1. In dogs with widely open pneumothorax, the association of inspiratory and expiratory movements of the thorax and distention and collapse of the lungs results from inhibition of the respiratory movements during each insufflation of the lungs.

2. This association cannot be fully explained by inhibitory impulses arising from the distended lungs, for their state as shown in part I of this paper is able to occasion apnea only under certain conditions.

3. Motion of the lungs or, more properly, one of the factors of motion, stretching of lung tissue, changes in alveolar tension or pulmonary blood pressure, motion of the air in the bronchial tubes, appears necessary for this association.

BIBLIOGRAPHY

- BREUER, J. 1868. Sitzungsab. d. K. Akad. d. Wissensch. Math.-naturw. Kl. ii, lviii, Wien.
- HALDANE, J. S. 1922. *Respiration*. Yale Univ. Press. New Haven.
- LOCKENBERG, E. 1873. Verhandl. d. Physikal-Med-Gesellsch. in Würzburg. Neue Folge iv, 239.
- GUTTMANN, P. 1875. Arch. f. Anat., Physiol. u. wissenschaft. Med., p. 500.
- HEAD, H. 1889. Part I. Journ. Physiol., x, 1.
- CHRISTIANSEN, J. AND J. S. HALDANE. 1914. Journ. Physiol., xlviii, 272.
- FILEHNE, W. 1879. Arch. f. exp. Path. u. Pharm., x, 442, xi, 45.
- KNOLL. 1925. Quoted by G. BAYER. Handb. d. norm. u. path. Physiol., ii, 455.
- WIELAND, H. 1916. Arch. f. exp. Path. u. Pharm., lxxix, 95.
- VAN SLYKE, D. D. AND J. M. NEILL. 1924. Journ. Biol. Chem., lxi, 523.
- HASTINGS, A. B. AND J. SENDROY, JR. 1924. Journ. Biol. Chem., lxi, 695.
- HEYMANS, J. F. ET C. HEYMANS. 1927. Arch. Internat. d. Pharmacodyn. et de Therap., xxxiii, 273.
- HEYMANS, C., J. J. BOUCKAERT, ET L. DAUTREBANDE. 1930. C. R. de la Soc. de biol., cv, 881.
- TRAUBE, L. 1846. Beit. z. exp. Path. u. Physiol., i, 65; ii, 91.
- HESS, W. R. 1930-31. Pflüger's Arch., ccxxvi, 198.

REFLEXES IN SPINAL STANDING OF THE CAT¹

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It has been shown that chronic spinal cats exhibit extensor tonus which is sufficient to support the weight of the body in the standing posture (Ranson and Hinsey, 1930). Observations made upon intact limbs (Ranson and Hinsey, 1930; Hinsey, Ranson and Zeiss, 1931) suggested that this spinal standing was subserved not only by the shortening reaction (Sherrington, 1909) and the stretch reflex (Liddell and Sherrington, 1924, 1925), but also by the positive Stütz or supporting reaction (Rademaker, 1926, 1931; Magnus, 1926; Schoen, 1926; Pritchard, 1926). Fulton (1926) has pointed out that there is no reason for considering the shortening reaction as other than a manifestation of the stretch reflex. In this paper the two will be considered under the term stretch reflex.

The positive Stütz reflex consists of contraction of both extensors and flexors of the leg, elicited by applying pressure to the pads of the toes, similar to that of the floor on the foot in standing. The entire limb is fixed in extension so that it resists flexion at all joints. It was seen by Rademaker (1926) in decerebellate dogs and has been studied by Magnus (1926), Schoen (1926), and Pritchard (1926). It was found in thalamic animals but Schoen could not obtain it in spinal animals, although there was a suggestion of it when the extensor tonus was exaggerated by tonic neck reflexes. In addition to this positive reaction, Magnus and Schoen described a negative Stütz reflex which was elicited by passive plantar flexion of the toes. When this was done, the extensor tonus relaxed and the resistance to passive flexion disappeared. It is seen particularly well developed in the decerebrate animal, where it is difficult to demonstrate the positive reaction due to the exaggerated extensor tonus.

In as much as Schoen (1926) did not see the positive Stütz reflex in acute spinal animals, doubt is cast upon the validity of our observations as to its presence in the intact limbs of chronic spinal preparations. Either the spinal standing which we have seen is dependent upon the presence of the stretch reflex, or the "neural balance" in Schoen's preparations was not tilted to the extensor side sufficiently to permit the demonstration of

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the Stütz reflex. An endeavor to answer this question has been made in the observations described in this report, where we have analyzed the responses in isolated muscles acting at the knee-joint in chronic spinal animals.

METHOD. Eighteen chronic spinal cats were prepared by transecting in the lower thoracic segments according to the method described by Ranson and Hinsey (1930). In our post-operative treatment, we did not inject salt solution subcutaneously, but forced milk by mouth twice daily. The rectum and bladder were evacuated daily by manual pressure on the abdomen. We had no infections and sixteen of the eighteen animals could stand on the day of the experiment. The animals were kept from one to thirteen days following the transection before the final experiment was performed. Observations were made daily during this period to determine the ability of the animals to stand. They were supported in a hammock made of muslin, which contained four holes through which the legs hung, and were examined for the presence or absence of the positive Stütz reflex, ipsilateral flexion, crossed extension and crossed flexion.

Kymograph records were taken of the isolated extensor quadriceps femoris and the flexor semitendinosus. The animals were first decerebrated by the anemic method of Pollock and Davis (1924). The decerebrations were successful in all but one of the preparations, and the animals remained in excellent condition throughout the course of the experiments. The isolation of the muscles was completed by resecting the insertions of all of the muscles upon the proximal end of the femur, by section of the nerve supply to all the thigh muscles with the exception of the two isolated ones, and by dissecting and denervating the rectus femoris. The animal was then placed in the hammock and the right hind limb was securely fixed so that only the contractions of the two muscles would be transmitted to the levers. This was done by transfixing the distal end of the femur with a drill which was held in place by two upright supports which were adjustable, being fastened by screw nuts at the bottom and by U-clamps into the frame at the top. The proximal end of the femur was clamped as was also the ischium of the pelvis. In some of the experiments a clamp was applied to the fibula just above the ankle-joint. The frame supporting the hammock was screwed to the laboratory table so as to be immovable. The contractions were recorded on the kymograph by means of isotonic levers, with rubber bands used in place of weights.

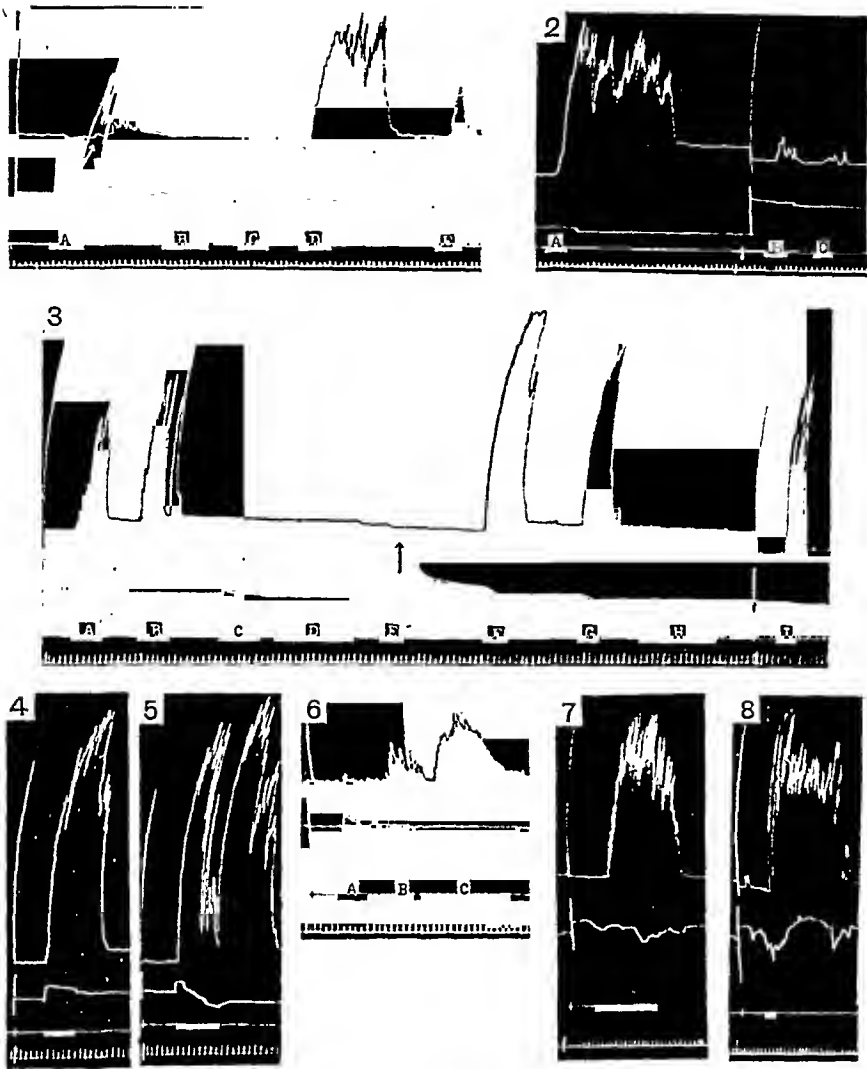
Reflex responses were obtained by applying normal stimulation to the ipsilateral and contralateral hind limbs. For example, it was possible to stimulate for the positive Stütz reflex by gently applying pressure to the pads of the toes and to observe the responses in the thigh muscles. Later on, in some of the experiments, the contralateral tibial nerve at the ankle, the saphenous and the sciatic nerves were exposed and stimulated with

induction shocks applied through shielded platinum electrodes. The ipsilateral tibial nerve at the ankle was also exposed and stimulated. A Harvard coil was used with interruptions at the rate of 50 per second, with both make and break shocks present. The current in the primary was about 2 amperes and the position of the secondary varied from 13 to 6 cm.

There are several advantages to this method. It makes possible a comparison of normal with electrical stimulation. While it is possible to control electrical stimulation in a quantitative way, it is at best unsatisfactory in eliciting reflex responses because the individual fibers in the nerve are activated without respect to the type of sensation they mediate, their normal frequency, or their reflex sign. With the animal in the upright position supported in a hammock, the posture is similar to the normal one and it is as close an approximation as it is possible to obtain and still record the contractions of the muscles. It has been pointed out (Ranson and Hinsey, 1930) that there is a sensitive inhibitory reflex from the skin of the back acting on the positive Stütz reflex, and for this reason an animal on its back would be unsuited for our purpose. Furthermore, the pressure of the hammock upon the skin of the abdomen and groin was found to reinforce the resistance to passive flexion of the leg, a fact which should contribute to the demonstration of the Stütz reflex if it were present in these animals.

RESULTS. *The positive Stütz reflex.* This reflex was not present in its entirety in our preparations for it was not possible to obtain cocontraction of the extensors and flexors acting at the knee-joint by applying gentle pressure to the pads of the toes. This absence of cocontraction was seen at times in the experiments when ipsilateral flexion, crossed extension (figs. 1 and 3), ipsilateral extension and the stretch reflex were demonstrable. Therefore it cannot be said that its absence is due to the poor reflex condition of our animals. Different types of stimulation were utilized, i.e., the slightest pressure to the pads of the toes, greater pressure so as to put the gastrocnemius on the stretch, and maximum passive extension of the gastrocnemius with dorsiflexion of the toes. The paws were also grasped in the position of the positive Stütz and the limb was passively flexed and extended in rapid succession. Bilateral simultaneous positive Stütz was also tried in an endeavor to elicit the response. The responses in the quadriceps and semitendinosus were either entirely negative, ipsilateral flexion was present or ipsilateral extension was obtained, but they never cocontracted in our preparations where there was complete dissection and fixation. Sometimes following ipsilateral flexion, an extensor rebound was seen, both following Stütz positive stimulation and pinching the pad (fig. 1 A).

The importance of complete fixation cannot be emphasized too much. In some of our earlier experiments, we saw cocontraction and believed that



Figs. 1-8. Tracings recorded by right quadriceps (above) and semitendinosus (below). Isotonic contractions. Time in seconds.

Fig. 1. Spinal cat 260, with transection at T 10, five days after operation. A. Ipsilateral flexion from pinching foot. B and C. Absence of positive Stütz reflex on gentle dorsiflexion of toes. D. Crossed extension on pinching left toes and at the same time applying gentle dorsiflexion to the toes of the right foot. E. Crossed extension on pinching left toes without any concomitant stimulation of the right limb.

Fig. 2. Spinal cat 260. A. Crossed extension on pinching left toes and applying gentle dorsiflexion to toes of right foot. B and C. Crossed extension on pinching left toes without any concomitant stimulation of the right limb.

Fig. 3. Spinal cat 270, with transection at T 10, two days after transection. A. Crossed extension on pinching left toes. B. Crossed extension on very strong pinching of left toes. C and D. Stütz positive stimulation to right toes. E. Ipsilateral flexion on pinching toes of right foot. F. Crossed extension on pinching skin about knee-joint. G. Crossed extension on very strong pinching of skin about knee-joint.

we had demonstrated the Stütz reflex in its entirety. However on examination we found that the upright supports were very slightly movable. We then fastened them at the top with U-clamps and the cocontraction was no longer obtained.

Ipsilateral extension. However the absence of cocontraction does not necessarily mean that stimulation of the pads of the toes is without effect upon them in the standing posture. It was possible to demonstrate in five of the animals a definite response in the extensor quadriceps from Stütz positive stimulation. With very slight pressure upon the toe-pads, ipsilateral extension was elicited (fig. 7). Figure 8 illustrates a crossed extension response in this same preparation in which there is a prolonged extensor contraction which appears as an extensor rebound. While ipsilateral extension could be seen very well without previous stimulation, it could also be demonstrated when the stimulation was applied either during the phase of relaxation of a crossed extensor response or immediately after the muscle had relaxed following such a response. In figure 6, Stütz positive stimulation was applied at A, crossed extension was elicited at B, and ipsilateral extension at C. The response at C was obtained by applying slight pressure to the toes of the ipsilateral limb and the contraction is greater in amplitude than the crossed extension. The presence of "direct" as well as "indirect spinal induction" (Sherrington, 1906) may be explained by the assumption that the motor neurones which take part in the crossed extension reflex probably also participate in the ipsilateral extension reflex. "Direct spinal induction" might be explained as due to a central summation and "indirect spinal induction" to an increased excitatory state which is residual following the crossed extension reflex. This latter explanation was given by Creed, Denny-Brown, Eccles, Liddell and Sherrington (1932) to explain the greater readiness of the reflex centers to respond to the stretch reflex after crossed extension. We could obtain no evidence of "negative successive induction" to the Stütz positive reflex following ipsilateral flexion.

H. Stütz positive stimulation again applied. I. Crossed extension on moderate pinching of the toes.

Fig. 4. Spinal cat 270. Crossed response caused by electrical stimulation of the left saphenous nerve. Harvard coil at 6 cm., rapid faradic, about 2 amperes in the primary.

Fig. 5. Spinal cat 270. Crossed response due to stimulation of the left tibial nerve at the ankle. Coil at 10 cm.

Fig. 6. Spinal cat 259, with transection at T 10, two days after operation. A. Gentle pressure applied to right foot-pads. B. Crossed extension on pinching left toes. C. Gentle pressure applied to right foot-pads, showing ipsilateral extension.

Fig. 7. Spinal cat 262, with transection at T 9, five days after operation. Ipsilateral extension on very gentle pressure applied to right foot-pads.

Fig. 8. Spinal cat 262. Crossed extension on pinching left toes.

The ipsilateral extension which was obtained was of a different nature than the extensor thrust which Sherrington (1906) saw in spinal dogs. While his response was of a transitory nature, barely lasting one-half second, the ipsilateral extension which we have seen has lasted several seconds (figs. 6 and 7). It was difficult to obtain, appeared late following the decerebration and occurred in only four experiments. We have never been able to produce pure ipsilateral extension in the spinal animal with electrical stimulation. We have seen cocontraction of the extensors and flexors with electrical stimulation but never extension alone. This is in agreement with the observations of Sherrington and Sowton (1911), Brown and Sherrington (1912) and Ranson and Hinsey (1931). Pinching of the toes in the spinal animal may cause cocontraction while the usual response to both weak and strong stimulation on pinching is ipsilateral flexion and extensor relaxation.

Facilitation of crossed extensor responses. One of the most interesting observations was the facilitation of the crossed extensor responses by applying Stütz positive stimulation to the ipsilateral foot. In figure 1 D, the crossed extension was produced by pinching the contralateral toes with simultaneous Stütz positive stimulation of the ipsilateral limb. The response is quite large in amplitude and long in duration as compared with the contralateral extension (fig. 1 E) obtained without any concomitant pressure on the ipsilateral toe pads. This same comparison may be made in figure 2 between A on the one hand and B and C on the other. This is open to the objection that there is a lack of uniformity of stimulation in pinching the contralateral toe to produce the crossed extension. We endeavored to keep the stimulation as uniform as possible under the conditions of the experiment. Admitting that there was some difference in stimulation, we were convinced that there was no question but that the crossed extension responses were increased in amplitude and duration by applying concomitant Stütz positive stimulation to the limb in which the response was recorded. This may be explained by assuming that the afferent impulses set up by pinching the contralateral toes and those produced by pressure to the ipsilateral toe-pads play upon the same motor neurones or spinal centers and that there is a resulting summation. A similar facilitation to the stretch reflex by the Stütz positive stimulation was observed by Denny-Brown (1929) in the decerebrate animal.

Crossed responses. In this series, crossed flexion was seen to be very well developed in the intact limbs before dissection in only four animals on strong pinching of the toes. In six others, it was present but very difficult to obtain, and then as a rule only at the ankle-joint. In the dissected limb, it was obtained in only three preparations, and in two of these only early in the progress of the experiment. The responses to normal stimulation in the isolated muscles was predominantly extensor in type

with concomitant flexor relaxation, although slight flexor cocontractions were seen on strong stimulation and flexor rebounds were present occasionally.

On electrical stimulation of the tibial at the ankle, saphenous and sciatic nerves, again the responses were extensor, both with weak and strong stimuli (13 cm. and 6 cm. separation of the secondary coil). We did not see the nerve reversals which were present in the gastrocnemius and tibialis anterior muscles in other spinal preparations (Hinsey, Ranson and Doles, 1930). In only one of the eight animals in which electrical stimulation was applied did we observe pure crossed flexion. In the remaining seven when cocontraction was present even on strong stimulation, the response in the flexors was small in comparison with that of the extensors in the same response and with that of the ipsilateral flexion reflex in the same animal.

Strong pinching of the toes is shown in figure 3 B to produce crossed extension and extensor rebound with no flexor participation. On the other hand, electrical stimulation (10 cm. coil separation) of the tibial nerve at the ankle is seen in figure 5 to elicit a cocontraction with inhibition during stimulation and a marked postexcitatory extensor rebound. In figure 3 G, it is seen that there is an absence of cocontraction on strong pinching of the skin about the knee-joint, while figure 4 shows crossed extension with flexor cocontraction from electrical stimulation (6 cm. coil separation) of the saphenous nerve in the same preparation. Comparisons of this nature, together with similar ones in the ipsilateral responses to which mention has been made, show that there is some difference in the responses obtained from normal and electrical stimulation. This is due probably to the massive stimulation of the nerve fibers without respect for their reflex sign and also to the fact that the electrical stimulation is not of a normal frequency.

The more common occurrence of the extensor crossed responses in this series may be attributed to one of two causes or to both. In the first place, the conditions of our experiments favored a "neural balance" tipped toward the extensor side. Sixteen of eighteen of these animals could support the weight of their bodies in standing on their hind limbs before the dissection was started. They were placed upright in the hammock free from extensor inhibiting influences from the back and in a posture favorable to extensor reflexes. In the second place, in this series we were dealing with the extensor quadriceps and the flexor semitendinosus, while in our previous experiments we were recording responses in the extensor gastrocnemius and the flexor anterior tibialis.

We found that the crossed responses developed a great deal as the experiments progressed. For example, cat 270 was decerebrated at 11:15 a.m. At 1:45 p.m., no crossed responses could be obtained. At 2:05, stimulation of the toes was negative for the contralateral side but pinching

the skin over the knee-joint gave crossed extension. At 2:50, the same results were obtained. At 4:20, stimulation of the skin of the toe gave a small crossed extension, that of the skin of the knee a very good one. At 4:40 and 5:00, very good crossed extensions were elicited from both regions. This was seen in other experiments and illustrates the fact that crossed responses recover much slower than the ipsilateral ones (flexion) which were present at 1:45 in good amplitude. Furthermore it shows that the recovery for crossed reflexes differs in the sensory field about the knee-joint from that in the toes.

COMMENT. Schoen (1926) pointed out that there are three essential differences between the positive Stütz reflex and the stretch reflex. 1. Magnus (1926) showed that two types of stimuli may produce it, *a*, proprioceptive elicited by stretching the plantar flexors of the toes, and *b*, exteroceptive by touching the pads of the toes. The stretch reflex is produced only by proprioceptive stimulation. From our experimental evidence, we cannot say what type of stimulation was active in producing ipsilateral extension. 2. Stimulation of the foot in the Stütz reflex calls forth contractions of the muscles of the entire limb, while stretching of the extensor muscle in the stretch reflex elicits a response only in that muscle and there is no spread to other muscles of the limb. The ipsilateral extension we have seen represents a spread from the sensory field in the pads of the toes to the extensor quadriceps acting at the knee-joint. 3. Flexors and extensors as well participate in the positive Stütz reflex while the stretch reflex is present only in the extensor muscles (Liddell and Sherrington, 1924, 1925; Schoen, 1926). We have not seen cocontraction from the Stütz positive stimulation but we have been able to elicit individual contractions of both from pressure on the foot-pads. Thus only a portion of the Stütz pattern could be demonstrated in spinal animals.

We have no observations which are contradictory to the statement of Sherrington (1924), "Elicitation by gravity of the stretch reflex of the limb extensor suggests itself as a basic factor in this static geotropic reflex of standing." These experiments do show however that in addition to the stretch reflex, there are reflexes from the pads of the feet which may reinforce the extensor tonus of the spinal animal. The stretch reflex seemed to be much more resistant to the experimental procedure and was found to be present when the extensor facilitating mechanisms from the foot were not demonstrable. The reinforcement of the crossed extension reflex would be of great value in the extensor phase of the step. Furthermore, ipsilateral extension from the foot should add to the resistance to passive flexion caused by the stretch reflex in standing. This in turn may very well explain the observation that the resistance to passive flexion at the knee-joint from the position of the positive Stütz reflex stimulation is greater than that felt when the force is applied above the ankle in the chronic spinal animal.

In the intact animal, it is highly probable that impulses from the stretched extensor muscles and from the pads of the feet are both active in spinal standing.

It is of interest that Foerster (1927) reports having seen the Stütz positive reflex in two cases of spinal transection in man. Contrary to Rade-maker's opinion (1931), we have seen what we have taken to be the Stütz positive reflex in babies, one 2 weeks old, another 8 weeks, and still another 16 weeks old. While Chaney and McGraw (1932) did not look for this reflex, they report that 43.5 per cent of newborn babies are able to support their weight momentarily in the standing posture. This momentary standing may have been subserved in part at least by the Stütz positive reflex.

In the spinal as well as in decerebrate animals, there may be exceptions to the law which says that the ipsilateral response is flexion and the crossed one extension. These exceptions in ipsilateral responses require normal stimulation to bring them out and they are difficult to elicit in the spinal animal. It would seem that the spinal cord in spinal animals possesses nearly all, if not all, of the essential mechanisms for subserving tonic activity of a certain degree. While impulses from higher centers may regulate and reinforce the spinal mechanisms and add to them equilibration, their absence in the spinal animal does not make the spinal cord an altogether different field of activity for reflex mechanisms nor deprive it of its ability to subserve a utilizable tonus maintenance in the cat.

SUMMARY

In a series of eighteen chronic spinal cats transected in the lower thoracic segments, decerebrations were performed, using the anemic method. The extensor quadriceps femoris and the flexor semitendinosus were isolated by dissection and the animal was placed upright in a hammock. With the right hind limb securely fixed, kymographic tracings were made of the reflex responses in these muscles. In the same animal, it was possible to apply normal stimuli to the ipsilateral and contralateral limbs and also to use electrical stimulation to the various nerves.

The Stütz positive reflex (positive supporting reaction) with cocontraction of the flexors and extensors of the knee was not elicited on stimulation of the pads of the foot. In some preparations, a portion of the pattern was present as an effect on the extensor responses of the quadriceps, either an ipsilateral extension or a facilitation of the crossed extensor reflex, both in amplitude and duration. At times in the presence of a well-developed stretch reflex in the gastrocnemius, no response could be obtained from Stütz positive stimulation; at other times ipsilateral flexion was seen.

Crossed flexion was observed but the crossed responses to normal and electrical stimulation were generally extensor in type in these muscles acting at the knee-joint.

BIBLIOGRAPHY

- BROWN, T. GRAHAM AND C. S. SHERRINGTON. 1912. *Journ. Physiol.*, xlv, 125.
- CHANNEY, L. B. AND M. B. MCGRAW. 1932. *Bull. Neurol. Inst. of New York*, ii, 1.
- CREED, R. S., D. DENNY-BROWN, J. C. ECCLES, E. G. T. LIDDELL AND C. S. SHERRINGTON. 1932. *Reflex activity of the spinal cord*. London.
- DENNY-BROWN, D. 1929. *Proc. Roy. Soc., Series B*, civ, 252.
- FULTON, J. F. 1926. *Muscular contraction and the reflex control of movement*. Baltimore.
- FOERSTER, O. 1927. *Arch. f. Psychiat.*, lxxxi, 706.
- HINSEY, J. C., S. W. RANSON AND E. A. DOLES. 1930. *This Journal*, xcv, 573.
- HINSEY, J. C., S. W. RANSON AND F. R. ZEISS. 1931. *Journ. Comp. Neurol.*, liii, 401.
- LIDDELL, E. G. T. AND C. S. SHERRINGTON. 1924. *Proc. Roy. Soc., Series B*, xvi, 212.
1925. *Proc. Roy. Soc., Series B*, xevii, 267.
- MAGNUS, R. 1926. *Deutsch. Zeitschr. f. Nervenheilk.*, xciv, 141.
- POLLOCK, L. J. AND L. DAVIS. 1924. *Arch. Neurol. and Psychiat.*, xii, 288.
- PRITCHARD, E. A. B. 1926. *Pflüger's Arch.*, cxxiv, 148.
- RADEMAKER, G. G. J. 1926. *Deutsch. Zeitschr. f. Nervenheilk.*, xciv, 144.
1931. *Das Stehen*. Berlin.
- RANSON, S. W. AND J. C. HINSEY. 1930. *This Journal*, xciv, 471.
1931. *Arch. Neurol. and Psychiat.*, xxvi, 247.
- SCHOEN, R. 1926. *Pflüger's Arch.*, cxxiv, 21, 48.
- SHERRINGTON, C. S. 1906. *The integrative action of the nervous system*. New Haven.
1909. *Quart. Journ. Exper. Physiol.*, ii, 109.
1924. *Nature*, cxiii, 732, 892, 924.
- SHERRINGTON, C. S. AND S. C. M. SOWTON. 1911. *Proc. Roy. Soc., Series B*, lxxxiii, 435.

CONTINUOUS PANCREATIC SECRETION

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At present it is generally accepted that pancreatic juice is not secreted at all, or at best in only small amounts, during fasting or in the interdigestive periods. Since the work of Claude Bernard, Pawlow, and Bayliss and Starling, the conviction that entrance of food into the digestive tract is necessary to start the pancreatic secretory process has been so strong that the evidence reported by various investigators suggesting a continuous secretion has been overlooked.

Continuous secretion was noted but held to be pathological by Claude Bernard (1), Pawlow (2), Jablonski (3), Walther (3), Babkin (4), (Babkin (5) expresses a somewhat divergent point of view), Tonkich (7), Elman and McCaughan (11). Data or opinions favoring continuous secretion are given by Bylina (6), Ivy and Farrell (8), Ivy (9), Dodds and Bennet (10). Evidence for continuous secretion in various species is also available: comparative review (13), cats (12), rabbits (14), man (12, 15, 16).

In the following study we present data which indicate that pancreatic secretion in dogs is also continuous during fasting.

TECHNIQUE. In order to observe the flow of pancreatic juice we used the method described originally by Rous and McMaster (17) in their studies on biliary secretion. The same technique was adopted by Elman and McCaughan (11) in their studies on the pancreas. This method permits the collection of the total output of the pancreatic juice under most favorable conditions and without bacterial contamination. It differs from the Pawlow fistula in that the minor duct is ligated, thus cutting off all communication with the intestine. It also makes unnecessary the insertion of a cannula to dilate the cicatrizing end of the duct.

Under ether anesthesia, and with aseptic precautions, pancreatic fistulas were established in a series of large male dogs. The major pancreatic duct was isolated first. Then the minor duct was ligated and divided, and the portion of the pancreas between the two ducts was dissected free from the intestine, thus assuring destruction of other accessory ducts in this area. A cannula attached to a rubber U tube was inserted into the major duct and then the latter was divided close to the duodenum. The small

opening in the intestine left by the distal segment of duct was closed by interrupted inversion sutures. A piece of omentum was drawn through the space left between the pancreas and the duodenum and fixed to the latter by sutures in order to prevent the reestablishment of communications between the ends of the divided ducts. The rubber tube was brought out through the abdominal-wall by means of a stab wound just below the costal margin, and the free end was connected to a small glass T-tube which led to a rubber collecting bag. The whole apparatus was held in place by a protective dressing. The bag was emptied at intervals of usually 24 hours and the amount of secretion was measured. The animals recovered rapidly from the effects of the operation. For the first two days after the establishment of the fistula the secretion usually consisted of a small amount of very mucinous material. Thereafter the daily output varied between 200 cc. and 750 cc. and represented essentially normal juice of fairly constant composition. Some of the animals died at the end of 8 days with symptoms associated with acidosis and dehydration. Vomiting was not a pronounced symptom. All dogs used for long time experiments received daily injections of NaCl and NaHCO_3 to prevent dehydration and acidosis. They all lost weight, sometimes up to 38 per cent. At autopsy accessory communications between the pancreas and duodenum were looked for, but were never found. The pancreas showed gross and microscopic evidence of shrinkage of the parenchyma, which was probably associated with the loss of fat. The weight of the pancreas decreases during inanition very nearly in proportion to the body weight (18). Diminished turgidity due to lessened resistance to outflow may also be a factor. Anrep (19) has shown by oncometric measurements considerable variation in volume of this organ dependent on outflow. In most instances there was neither evidence of atrophy due to obstruction of the ducts nor of inflammatory changes.

For observations on spontaneous secretion during fasting, animals were deprived of food for periods varying from a few hours to 72 hours before the experiment was started. All precautions were taken to avoid the possible influence of conditioned reflexes upon secretion and the observations were made in a room where they never saw nor received food. They were trained to lie quietly and often slept while the experiments were in progress.

The rate of secretion was studied by connecting 5 cc. pipettes graduated in 0.02 cc. to the rubber outlet tube in a horizontal position, and making readings at suitable time intervals. Observations were made over periods extending from one hour to 3 hours, or even longer, and specimens were collected for analysis.

The "secretory pressure" (20, 21, 22) was determined by measuring the maximum height attained by the column of juice when the duct system was

connected with a manometer tube of 2 mm. bore. A column 100 mm. in height was equivalent to 0.7 cc. of pancreatic fluid. The manometer readings were taken as approximations of secretory pressure.

EXPERIMENTAL RESULTS. *Continuous pancreatic secretion during fasting and the temporary acceleratory effect of secretin.* The following table represents a typical example of continuous pancreatic secretion in an ani-

TABLE 1
Dog 34. Continuous secretion of pancreatic juice
Fistula established October 17, 1930

DATE	LENGTH OF PERIOD	CC. COLLECTED	NUMBER OF CC. PER MINUTE	REMARKS
	<i>hours</i>			
Oct. 18.....	24	None		
Oct. 19.....	24	None		
Oct. 20.....	24	180.0	0.063	
Oct. 21.....	24		0.063	
Oct. 22.....	24	75.0	0.052	
Oct. 23.....	24	130.0	0.090	
Oct. 24.....	24	200.0	0.139	average 0.143
Oct. 25.....	24	210.0	0.146	
Oct. 26.....	24	120.0	0.083	
	<i>minutes</i>			
	10	1.28	0.128	Dog fasting 72 hours. No water allowed for 3 hours before readings made
	10	0.428	0.043	
	10	0.856	0.086	
	10	0.286	0.028	
	10	0.143	0.014	
	10	0.143	0.014	
Oct. 28.....	10	0.214	0.021	
	10	0.143	0.014	
	10	0.643	0.064	
	10	0.572	0.057	
	10	0.856	0.086	
	10	1.07	0.107	
	Average		0.055	

0.055 cc. per minute is equivalent to 79.5 cc. per 24 hours, which is about 26 per cent of the amount secreted before fasting.

mal fasted 72 hours. The average rate of secretion after fasting 72 hours was about one-fourth of the average rate in 24 hour secretions before fasting. We look upon the 24 hour collections in dogs that received food as being made up of the basal continuous secretion plus secretin juice.

In another dog, the rate of secretion decreased more or less continuously during 48 hours of fasting, coming to about one-third of the pre-fasting rate. This type of observation was repeated a great many times with the same result.

It may be well to point out at this juncture that the term "continuous" does not necessarily imply an uninterrupted stream of pancreatic juice during the interdigestive period; pancreatic secretory activity continues in the absence of food stimuli, the rate of flow varying from minute to minute and from hour to hour (see tables). As has been stated by other investigators, the secretion of the pancreas seems to be discharged in spurts when observed in a pipette (see Biedermann, 13). This is analogous to the discharge of urine by the kidney.

After establishing the basal rate of secretion during fasting, the influence of secretin was determined.

When 10 cc. of a solution of secretin, prepared according to the method of Weaver (23) and co-workers, were injected intravenously into a fasting animal the flow of pancreatic juice was increased temporarily. This acceleratory effect lasted about 10 minutes and then the regular fasting rate of secretion was resumed. Marked variations occurred in the rate of secretion in the preliminary period of observation. However, with secretin, the rate increased beyond these variations. The results with secretin in these experiments differ from those of Bayliss and Starling in so far as they represent an acceleration of flow rather than an initiation of secretion. Examination of the data of Bayliss and Starling's acute experiments shows that there was no secretion before the injection of secretin or after the effect of secretin had worn off. This was due to the fact that the observations were made while the animal was under the influence of an anesthetic and other factors which are discussed later.

A parallel study was made of the secretory pressure of the pancreas during fasting and after the ingestion of food. This was repeated on several dogs with similar results; for the sake of brevity we include here only a typical example (see fig. 1, dog 6). Here the maximum fasting pressure attained was 340 mm. One hour and 20 minutes after feeding the pressure curve rose to 390.

So far as we know these are the first figures recorded for secretory pressure of the pancreas in the unanesthetized animal under fasting conditions. While the fasting pressure is of about the same order of magnitude as the bile pressure (20, 21, 22) it rises distinctly after feeding or secretin injection. In this respect the pancreatic secretion differs from bile. The bile secretion pressure is the same before and after feeding (22). The rate of secretion, therefore, does not affect the "secretory pressure." The importance of these facts will be referred to below.

Does continuous secretion represent pathological hypersecretion? At this point it may be profitable to discuss the question whether the phenomenon which we have described is a pathological hypersecretion, as was maintained by the early investigators in this field, and by more recent workers including Babkin (4) and Elman and McCaughan (11). Claude Bernard

and Pawlow attributed continuous secretion to irritation by the cannula in the intubated duct and to infection. Careful dissection of the drainage system at autopsy in our animals revealed that the cannula always lay outside the pancreas, enveloped in a connective tissue sheath which replaced the small segment of duct which originally held the cannula; the latter never encroached upon the parenchyma or the intrapancreatic portion of the duct system. With regard to infection, careful microscopic examination of the pancreas, post-mortem, revealed no evidence of inflammation except for some connective tissue proliferation in the region

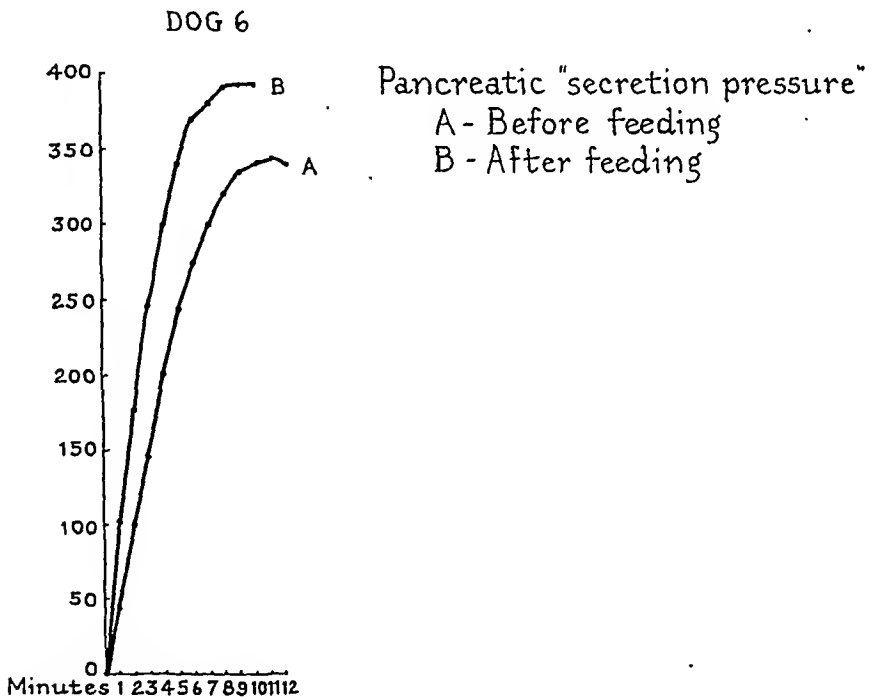


Fig. 1. Manometer readings in millimeters of pancreatic juice

of the dissection, which was done at the time of intubation of the major duct.

Babkin, on the basis of Walther's experiments, brought the continuous pancreatic secretion into relation with a gastric hypersecretion resulting from the pancreatic fistula and more recently Elman and McCaughan again advanced this point of view attributing the spontaneous pancreatic flow to a secretin effect, resulting from the lack of neutralization by the pancreatic juice of the exaggerated gastric secretion. Babkin in his extensive discussion of the subject puts all continuous secretion down as "pathological hypersecretion" and describes the experiences of Jablonski

and Walther in connection with the symptoms, with which we are now familiar, resulting from the *complete* loss of pancreatic juice. It should be noted that Babkin specifically states that in the dogs operated upon by the Pawlow technique the minor duct is left intact, i.e., communicating with the intestine. According to Babkin, conditions are "normal" only when there is a suitable distribution of flow between the duct leading to the intestine and the duct leading to the outside. In Jablonski's dogs there was evidently little or no flow into the intestine. Now since the flow to the outside was large and dehydration and other symptoms occurred, Babkin called this "pathological hypersecretion." He is quite right in saying that for purposes of long observation dogs must be used in which sufficient juice reaches the intestine, but when he calls complete diversion of juice to the outside "pathological hypersecretion," it seems he is begging the question.

The gastric hypersecretion as a possible factor is excluded by observations of Tonkich (7) and Bylina (6). Both observed continuous secretion independently of the possible effect of gastric HCl reaching the duodenum.

To further verify this point under our own working conditions, the following experiments were performed. A 2 per cent solution of NaHCO_3 was introduced into the stomach of a fasting secreting animal in amounts far beyond the quantity of alkali which could be supplied by the pancreas in order to neutralize the gastric contents. After the introduction of three successive portions of 50 cc. of 2 per cent NaHCO_3 with 1 gram of suspended CaCO_3 , the rate of flow was not affected.

Then an animal was prepared in the usual manner with a fistula and in addition provision was made for the reintroduction of the pancreatic juice into the duodenum a few inches below the ampulla. After secretion rate had been observed for a while, the juice was returned to the intestine from a drip apparatus in large quantities with the following results.

In two experiments 100 and 250 cc. of juice respectively were introduced, at a rate between 0.5 and 1.5 cc. per minute. The mean rate of flow from the pancreas was observed before, during, and after this procedure and was found to be undiminished. The figures were 0.120, 0.160, and 0.150 cc. per minute respectively. A similar experiment was performed, introducing the juice through a tube by mouth into the stomach rather than through an opening in the duodenum. In this dog the original rate varied between 0.253 and 0.155 cc. per minute. While the juice was being introduced the rates varied between 0.200 and 0.392 cc. per minute. Again there was no inhibition of flow.

Finally, an animal was prepared with a system of altercursive intubation as described by Elman and McCaughan (11), by means of which the pancreatic juice was continuously returned to the intestine through the biliary tract. Briefly, this consists of an intercommunicating system of

tubes leading from the pancreas to the gall bladder, arranged in such a way that the flow of fluid can be observed outside the body. By this procedure the pancreatic juice was allowed to enter the duodenum through the ampulla and neutralization of gastric HCl could continue as in the normal animal. Elman (24) has shown that the gastric hypersecretion which he reports as accompanying complete drainage to the outside does not occur under these conditions.

For the first three days after intubation, pancreatic juice was collected separately in a rubber bag, in order to determine whether the juice flowed freely. On the third day, 340 cc. of juice were collected in 24 hours. The bag was then removed and the juice allowed to flow into the intestine

TABLE 2
Dog 98. Altercursive intubation

Rate of pancreatic secretion in fasting animal after juice was continuously returned to intestine for 7 days.

DATE	LENGTH OF PERIOD	RATE PER MINUTE	REMARKS
	<i>minutes</i>	<i>cc.</i>	
Nov. 30.....	10	0.240	Dog fasting 24 hours
	10	0.175	
	10	0.130	
Dec. 1.....	10	0.140	Dog fasting 56 hours
	10	0.215	
	10	0.150	
	10	0.200	
	10	0.125	
	10	0.115	
	10	0.125	
	10	0.095	
	10	0.115	
	10	0.140	

through the biliary system. On the following day examination of the T-tubes revealed that they were both filled with clear pancreatic juice and no bile was visible. The outlet clamp upon the pancreatic side of the system was then released momentarily until a column of bile appeared in the tube on the biliary side of the system. The clamp was then re-applied and in about 20 minutes the bile had disappeared and was replaced by pancreatic juice. This preliminary observation indicated that the tube system was clear and that pancreatic juice was flowing through the biliary system.

Seven days later, the animal was deprived of food for 24 hours, water being allowed freely. During this time the juice had been made to flow

into the intestine. After the 24 hour fast, observations were made on the rate of pancreatic secretion. Then fasting was continued for the next 23 hours, water being given freely, during which time the juice again was allowed to flow into the intestine. The secretory rates are shown in table 2.

In order to avoid the possible criticism that the lack of alkaline pancreatic juice in the intestine during the collection periods set up an immediate secretion due to secretin production, the experiment was modified so

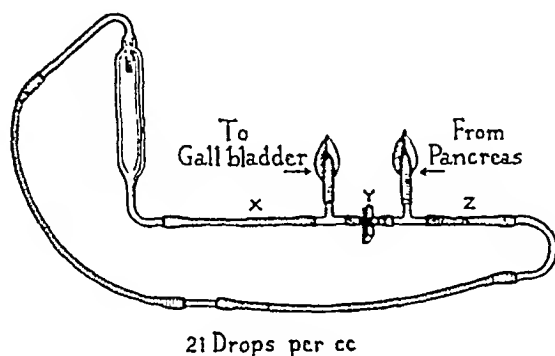


Fig. 2. Arrangement of drop counting bulb altercursive intubation

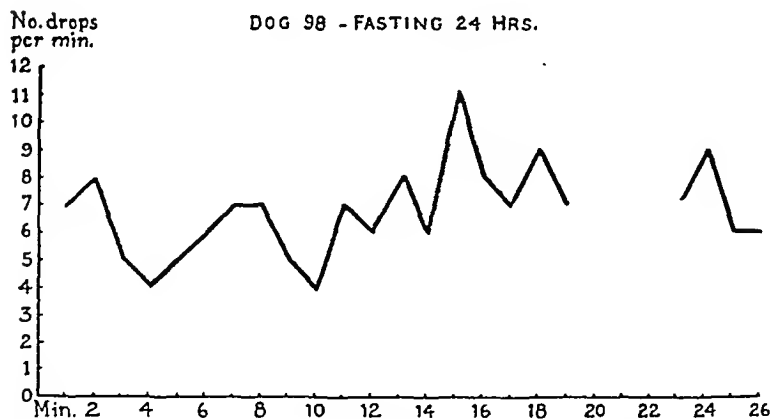


Fig. 3. Continuous secretion of pancreatic juice continuously returned to intestine.

that the return of juice to the intestine was never interrupted. A glass bulb with a glass tip leading into the top of it from the pancreas side and an outlet tube from the bottom of the bulb leading to the bile side of the altercursive system was introduced as shown in diagram (see fig. 2). This should give no possible opportunity for unneutralized HCl forming the postulated extra secretin and still the pancreatic flow continued. The results are shown in figure 3.

In order to show that the juice was flowing in this artificial system and

was being returned to the intestine during the intervals between observations, the pressure and resistance factors were studied. A T-tube manometer was inserted between the two duct systems in place of the glass bulb. When the clamp at *X* was applied with *Y* closed, the column of fluid gradually rose to a height of about 270 mm. (see B, fig. 4). This indicated the apparent pancreatic secretory pressure and corroborated our previous findings. The clamp at *X* was then removed and the column of fluid dropped abruptly, fluctuating between 160 and 110 mm. (see C to D, fig. 4). The sudden drop was due to the free entrance of juice into the

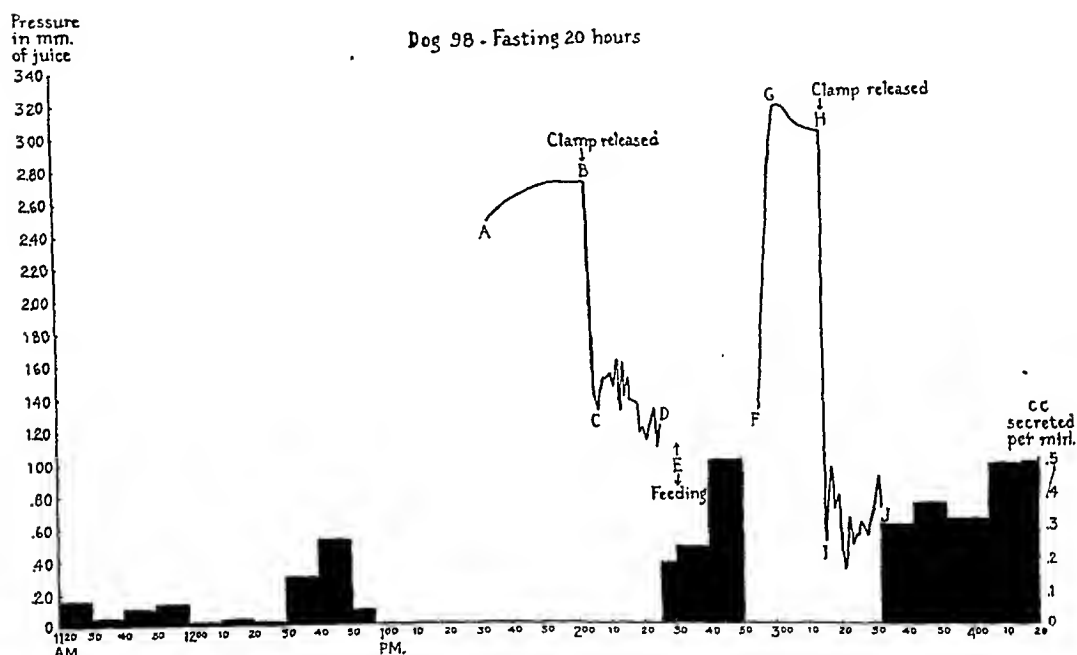


Fig. 4. Altercursive intubation (juice having been returned to intestine continuously for 11 days). Pancreatic secretory pressure and resistance—before and after food.

Lines in upper part of chart are pressure data with scale on left hand side. Blocks in lower part of chart give secretion rate with scale at right.

intestine and since the resistance at the sphincter of Oddi as determined by Elman and McMaster (25) varies between 100 and 120 mm. the column of fluid remained at that level temporarily. In another experiment done after 56 hours of fasting the column of fluid in the manometer rose to a height of 310 mm. and on opening of clamp, it fell promptly and fluctuated between 95 and 115 mm. The secretory pressure was always sufficient to insure entrance of juice into the intestine.

We are well aware that the conditions under which we made these observations differ somewhat from those of McMaster and Elman. Their

measurements refer to a biliary system in which the gall bladder had been removed, while in our animals the gall bladder was present and carried a cannula. But since our figures agree very well with those of McMaster and Elman, we feel that they may stand as they are. Further investigation along the ingenious and fruitful lines suggested by the two papers of McMaster and Elman (22, 25) would be very desirable. At this point it seems worth while to emphasize that in the discharge of secretion into the intestine the important point with regard to the pancreas is the continuous high pressure within the ducts, while in bile secretion the lowering of resistance to the outflow (22, 25) is a decisive factor.

Twenty-five minutes after giving one-half pound of raw meat the manometer was again attached and the pressure rose to 316 mm. (see F to G, fig. 4). When the clamp was released pressure fell to a level (I to J) lower than the previous level (C to D). This indicates a lowered resistance at the terminal portion of the common duct after feeding and agrees with findings reported by Elman and McMaster (25). The fluctuations in manometer readings after flow of juice into the gall bladder probably represent variable muscular tone in the intestinal wall. Elasticity of gall-bladder wall may also play a part. The fluctuations are not respiratory since all readings were taken as mean points of the respiratory movement which can be quite easily distinguished.

Serum amylase determinations showed that the flow of juice was not interrupted. Previous experiments have shown that when any obstruction occurs there is a distinct rise in blood amylase.

Summarizing this experiment, we may say that the flow of juice remained continuous in a fasting animal when provision was made for the continuous return of the fluid at its normal site of entrance into the intestine. This shows clearly that the phenomenon of continuous secretion is not the result of a constant secretin effect due to the gastric hypersecretion of HCl and the absence of neutralization of HCl by alkaline pancreatic juice. We should also note that when food is given to a continuously secreting dog, both rate and "secretion pressure" are increased. It is not very plausible therefore to assume that a "*pathological* hypersecretion" is subject to further increase by *normal* food stimulus.

Although in the dog it is practically impossible to measure the resistance at the end of the major pancreatic duct, due to its shortness, we may assume that the resistance is of the same order of magnitude as that at the sphincter of Oddi. It will be noted that when the secretory pressure reached its maximum and then, by releasing the clamp the pancreatic juice was allowed to enter the intestine, a column of fluid measuring from 100 to 150 mm. in height remained in the manometer. This column represented the pressure necessary to overcome the resistance at the terminal portion of the common duct and corresponded to the earlier observations made by

Elman and McMaster (22). The fact that the column of fluid fluctuated at this level indicated that there was a constant flow of juice secreted at a pressure always sufficient to overcome the resistance at the sphincter. Moreover, throughout the entire period of observation there was never any reflux of bile towards the pancreas as long as the system remained closed, showing that pressure in the pancreatic duct was always higher than that within the biliary tract. From this evidence we must conclude that there is no anatomical ground for the hindrance of continuous pancreatic secretion. It is also apparent that in animals which have gall bladders the normal flow of bile into the intestine can go on intermittently while through a common ampulla the secretion of pancreatic juice is continuous. Furthermore, we must concede that a continuous flow of pancreatic juice at a higher pressure than that in the biliary tract will effectually prevent a reflux of bile into the pancreatic duct as long as the pancreas is functioning normally.

Factors concerned in intermittent secretion described by previous investigators. When the methods of Claude Bernard, Heidenhain or Pawlow are used to create a pancreatic fistula, juice is obtained intermittently. During the inter-digestive period secretion is usually not observed and following the ingestion of a meal copious secretion is obtained. With these methods the minor duct is left intact. Under such conditions it is difficult to determine the quantity of juice which enters the intestine through the minor duct, especially in the fasting animal. Moreover, compensatory dilatation of the minor duct may occur during the interval required for healing of the transplanted major duct, at the site of repair. This must remain conjectural, because autopsy reports of the condition of the pancreas and its ducts were not made by those who employed this technique. However, during the inter-digestive phase, most of the secretion may escape into the intestine through the minor duct. During digestion or after the injection of secretin, the secretory pressure and rate of flow are increased and with the introduction of a cannula into the orifice of the fistula, part of the fluid escapes to the outside and part to the intestine. Accurate measurements of the total output are impossible, since the distribution of the juice between the intestine and the exterior depends upon the relative resistance in the two paths (see Babkin, p. 459), the ducts communicating within the gland. It was for this reason that dogs with Pawlow fistulas lived for long periods of time and remained in good condition whereas animals with *complete* fistulas succumb usually after one to three weeks. However, even when the latter dogs are moribund pancreatic secretion continues.

In the experiments of Bayliss and Starling (26), other factors were concerned in creating the impression that they were dealing with an intermittent secretion. Their observations were made in animals under anes-

thetia (A. C. E. mixture). Pancreatic secretion was obtained only after the intravenous injection of secretin. According to our observations this is due to several factors. We have observed repeatedly that the mere introduction of a cannula even with the gentlest manipulation causes a copious secretion of mucus which forms a plug in the duct or cannula and tends to obstruct the flow. Also, ether anesthesia inhibits the secretion of pancreatic juice. This is discussed in detail below. The influence of mucus formation upon pancreatic secretion was demonstrated in our experiments by the fact that relatively small quantities of fluid containing large amounts of mucus were secreted the first two days after operation and not until the third day was the regular rate of secretion attained and did the composition of the juice become normal.

Effect of ether on continuous secretion. One of the striking differences between our experiments and the acute type of experiment in which no spontaneous secretion is found is the use of an anesthetic. The indications in the literature that the pancreas is not indifferent to anesthetics led us to determine whether a dog secreting continuously would be influenced by being subjected to ether anesthesia. Ether anesthesia must, of course, be carefully distinguished from local application of ether in the small intestine. Katsch (28) and others use the stimulating action of ether locally applied to a test for external secretory function. Many other substances have a similar action. These agents probably work through the secretin mechanism.

The observations recorded in table 3 show that ether has a marked inhibitory effect upon the rate of pancreatic secretion. The effect manifested itself within the first 10 to 15 minutes after the induction of the anesthesia and lasted 7 hours. This is the typical result. It is well known that some dogs have a greater susceptibility to ether. In these only a light anesthesia can be maintained and the effect on the pancreas is less marked.

In this connection it is of interest to note that an animal can be maintained under a light surgical anesthesia with sodium amytal with only a slight inhibition of flow of pancreatic juice (see table 4).

A single injection of amytal in a dose of 25 mgm. per kilo was sufficient to induce surgical anesthesia and the rate of flow of juice was slightly slowed. When the injections were repeated more marked inhibitory effects occurred. A number of times when a normal dog which had fasted for 24 hours, was anesthetized with amytal, it was evident when the duodenum was opened opposite the papilla that spontaneous secretion was going on. With avertin anesthesia this was also found. If the surface was kept well moistened, secretion could be observed for quite a while. Slight irritation or the insertion of a cannula into the duct caused an outpouring

of mucus which quickly clogged the cannula and prevented the further flow of juice *at fasting secretory pressure*.

Effect of ether on serum amylase. So far we have shown that in a dog whose pancreas is cannulated by the Rous-McMaster technique the secretion is continuous. There is reason to believe that the condition of such an animal is more nearly normal than that of animals under anesthesia.

TABLE 3

Dog 87. Effect of ether anesthesia on continuous secretion of cannulated pancreas

	LENGTH OF PERIOD	AMOUNT SECRETED	RATE PER MINUTE	SERUM AMYLASE
		cc.	cc.	
1st preliminary period.....	9	4.0	0.444	80
2nd preliminary period.....	14	4.55	0.325	
3rd preliminary period.....	13½	4.10	0.300	
4th preliminary period.....	17	4.35	0.256	
Mean rate per minute for all 4 periods.....			0.331	
2:20 p.m. During induction of anesthesia..	15	1.65	0.11	
2:35 p.m. Under anesthesia lasting 1½ hrs.	14	0.65	0.05	
2:50 p.m.....	9	0.15	0.016	
3:00 p.m.....	7	0.00	0	
3:08 p.m.....	6	0.05	0.009*	
3:14 p.m.....	34	0.1	0.003*	100
3:47 p.m.....	48	0.1	0.0017*	100
4:37 p.m. Recovered from anesthesia 2 hrs. after induction.....	83	0	0	
6:00 p.m.....	60	3.5	0.058†	
7:00 p.m.	75	4.0	0.053†	
8:23 p.m.....	50	1.7	0.035†	
9:15 p.m.....	10	2.8	0.28	
9:25 p.m. 7½ hrs. after induction of anes- thesia.....	11	4.75	0.432	160

The various periods in the table were so chosen as to indicate changing rate of flow. The * indicates that the period contained several 0 per minute readings; † indicates that collection was made in rubber bag instead of pipette.

Before we conclude, however, that continuous secretion is a normal process in the dog similar to the continuous secretion of rabbits and of ruminants, we require some independent evidence that pancreatic activity is continuous in the unoperated animal. This information was obtained by studying the serum amylase of dogs subjected to ether anesthesia as reported in the following paper. Since it is generally conceded that no other

TABLE 4

Dog 89. Effect of amytal on continuous secretion of cannulated pancreas

Sodium amytal (Lilly) given intravenously

	LENGTH OF PERIOD	AMOUNT SECRETED	RATE PER MINUTE
	<i>minutes</i>	<i>cc.</i>	<i>cc.</i>
1st preliminary period.....	10	1.18	0.118
2nd preliminary period.....	10	1.67	0.167
3rd preliminary period.....	13	2.3	0.177
0.4 gram amytal....	10	0.4	0.04
	10	1.0	0.10
	10	1.3	0.13
	9	1.45	0.161
	13	1.5	0.115
	10	1.10	0.110
	10	0.9	0.09
	11	0.5	0.045
	10	0.63	0.063
	10	0.35	0.035
0.3 gram amytal.....	10	0.85	0.085
	10	0.61	0.061
	10	0.40	0.040
	7	0.54	0.054
	10	1.32	0.132
	10	1.10	0.110
	10	1.15	0.115
	10	0.93	0.093
	10	0.54	0.054
	10	0.33	0.033
0.2 gram amytal.....	10	0.28	0.028
	7	0.22	0.022*
	13	0.25	0.019
	15	0.33	0.022
	15	0.27	0.018
	13	0.30	0.023
	17	0.15	0.009*
	17	0.95	0.056
	11	0.35	0.032
	9	0.28	0.031
	15	0.42	0.028
	10	0.30	0.030
	25	0.70	0.028
	26	0.61	0.023
	<i>hours</i>		
	16	70	0.073

* Indicates periods containing several minutes with 0 cc. per minute reading.

procedure, except interference with the escape of juice from the gland will produce such effects, we can safely conclude that the influence of ether anesthesia on blood amylase is due to inhibition of excretion from the gland, while the continuous tendency to form juice is not impaired.

SUMMARY

1. In dogs whose pancreas is cannulated according to the Rous-McMaster technique secretion is observed to be continuous. Food or the injection of secretin temporarily increases the rate of flow. Secretin juice is secreted under a higher pressure than that of continuous secretion.

2. Anesthesia may completely inhibit the flow of pancreatic juice. With ether the effect seems to be more marked than with amytal.

3. Ether anesthesia increases the amylase level in the blood. This is explained on the basis of the double mechanism of formation and transport of pancreatic secretion. Ether abolishes the latter, thereby raising the blood amylase level through resorption of the enzyme.

4. In dogs with "altercursive fistulas" where there is admittedly no gastric hypersecretion and no lack of neutralization of gastric HCl, the spontaneous secretion can be demonstrated after 48 hours of fasting. The conclusion seems inevitable that continuous pancreatic secretion is a normal phenomenon and not "pathological hypersecretion."

BIBLIOGRAPHY

- (1) BERNARD, C. *Mémoires sur le pancréas*, etc. Paris 1856.
- (2) PAWLOW, I. *Vorlesungen*, Wiesbaden 1898. The work of the digestive glands. 2nd English ed., London, 1910.
- (3) Dissertations cited by BABKIN (4).
- (4) BABKIN, B. P. *Die äussere Sekretion der Verdauungsdrüsen*. Zweite Auflage, Berlin, 1928.
- (5) BABKIN, B. P. *Hdb. d. norm. u. path. Physiol.*, Berlin 1927, Vol. iii, p. 753.
- (6) BYLINA, A. S. *Pflüger's Arch.*, 1911, cxlii, 531.
- (7) TONKICH, A. *Pflüger's Arch.* 1924, ccvi, 525.
- (8) IVY, A. C. AND J. I. FARRELL. *This Journal* 1926, lxxviii, 325.
- (9) IVY, A. C. *Physiol. Reviews*, 1930, x, 282.
- (10) DODDS, E. C. AND T. I. BENNET. *Journ. Physiol.*, 1921, lv, 381.
- (11) ELMAN, R. AND J. M. McCAUGHAN. *Journ. Exp. Med.*, 1927, xlv, 561.
- (12) GAMBLE, J. L. AND M. A. McIVER. *Journ. Exp. Med.*, 1928, xlvi, 849.
- (13) BIEDERMANN, in WINTERSTEIN'S *Handbuch der vergleichenden Physiologie*. Vol. ii, p. 1384.
- (14) BAXTER, S. C. *This Journal*, 1931, xevi, 343.
- (15) LUCKHARDT, A. B., I. STANGEL AND F. C. KOCH. *This Journal*, 1923, lxiii, 397.
- (16) BABKIN, P. B. *Aussere Sekretion*, p. 484.
- (17) ROUS, P. AND P. D. McMASTER. *Journ. Exp. Med.*, 1923, xxxvii, 11.
- (18) JACKSON, C. M. The effects of inanition and malnutrition upon growth and structure. *Philadelphia*, 1925.
- (19) ANREP, S. V. *Journ. Physiol.*, 1915, xlix, 1; 1915, 1, 421.

- (20) HERRING, P. T. AND S. SIMPSON. *Quart. Journ. Exp. Physiol.*, 1909. ii, 99.
Proc. Royal Soc. Ser. B, 1907, lxxix, 517.
- (21) MANN, F. C. AND A. S. GIORDANO. *Arch. Surg.*, 1923, vi, 1.
- (22) McMASTER, P. D. AND R. ELMAN. *Journ. Exp. Med.*, 1926, xlv, 173.
- (23) WEAVER, M., A. B. LUCKHARDT AND F. C. KOCH. *Journ. Amer. Med. Assoc.*,
1926, lxxxvii, 640.
- (24) ELMAN, R. *Arch. Surg.*, 1928, xvi, 1256.
- (25) ELMAN, R. AND P. D. McMASTER. *Journ. Exp. Med.*, 1926, xlv, 151.
- (26) BAYLISS, W. M. AND E. H. STARLING. *Journ. Physiol.*, 1902, xxviii, 325.
- (27) ZUCKER, T. F., P. G. NEWBURGER AND B. N. BERG. *Proc. Soc. Exp. Biol. and*
Med., 1921, xxxix, 294.
- (28) KATSCH, G. AND L. v. FRIEDRICH. *Klin. Wochenschr.*, 1922, i, 112.

THE AMYLASE OF SERUM IN RELATION TO FUNCTIONAL STATES OF THE PANCREAS

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The literature on blood amylase has been very completely reviewed by Oppenheimer (1). With regard to serum amylase as an indication of the functional state of the pancreas there is good general agreement on many of the main features, certain rather important points, however, are still in dispute. It is conceded that the serum amylase is principally derived from the pancreas and that ligation of the ducts promptly leads to an increase. In carnivora all other organs are so much lower in amylase content than the serum, that any large increases are probably derived from the pancreas. We confine ourselves in this paper to conditions in the dog where salivary amylase is negligible. It is important to note (Anrep and others (2, 3, 4)) that pancreatic secretion comprises two mechanisms under separate control, the formation of juice and its transfer to the intestine. The enzyme enters the blood stream either directly from the gland or via the lymph channels. When the outflow is blocked, the serum amylase rises.

Some of the points which are not yet sufficiently clarified involve questions regarding the fate of serum amylase (excretion and possible destruction), reabsorption from the intestine and the effect on serum amylase of certain drugs which apparently affect pancreatic function.

METHODS. Serum amylase was determined by the Wohlgemuth (5) methods at pH 6.2 in the presence of a constant concentration of NaCl, as suggested by Michaelis (6). Over a range of 0.1 cc. to 5.0 cc. the action of a 1:100 dilution of a serum was determined in a reaction mixture of 10 cc., whose final starch concentration was 0.02 per cent. A stock solution containing 0.4 per cent soluble starch and 0.6 per cent NaCl will keep in a refrigerator for about a month unchanged. We make fresh solutions about every two weeks. For use this is diluted with an equal volume of M/20 phosphate buffer. Digestions are done at 37.5° for 30 minutes. The amylase of pancreatic juice and urine are determined similarly. A method of this kind, besides lacking in precision is also quite subjective when it comes to matching shades of colors in tubes. With some experi-

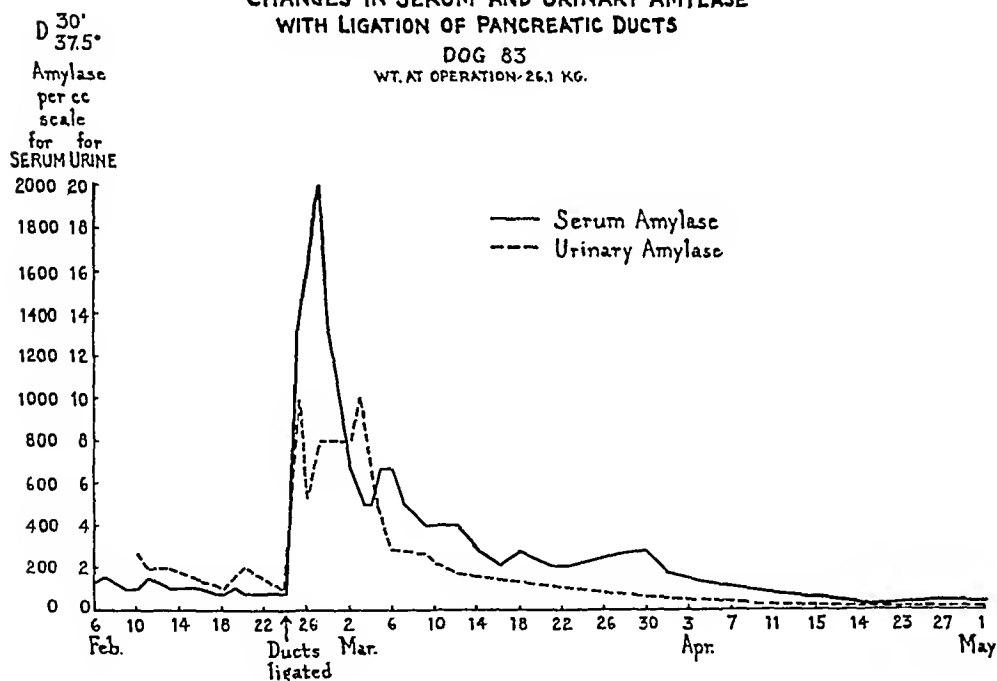
ence and careful repetition of tests at different dilutions when the first series does not show good demarcation, we believe that the results are within an error of about ± 5 per cent to 10 per cent. With the large differences usually recorded the method is satisfactory enough.

The figures recorded in this paper are calculated from the enzyme dilutions as conveniently used and are not intended necessarily to represent accuracy to the last digits. We use Wohlgemuth's designation D for amylase units.

For certain purposes lipase determinations have been included in this paper. Methods for serum lipase have been discussed by Cherry and

CHART I
CHANGES IN SERUM AND URINARY AMYLASE
WITH LIGATION OF PANCREATIC DUCTS

DOG 83
WT. AT OPERATION-26.1 KG.



Crandall (7). In the experiments recorded here, in order to compare results with those of Crandall and Cherry, the determinations were carried out essentially as described by them. It might be preferable to observe the precautions laid down by Willstätter (8).

SERUM AMYLASE AND URINARY AMYLASE AS INFLUENCED BY LIGATION OF THE DUCTS, PANCREATECTOMY AND FISTULA. *Ligation.* It is well established that ligation of the ducts increases the amylase in the serum and also in the urine. The curves (chart I) for serum and urinary amylase rise and fall in a rather parallel manner. An attempt to correlate quantitatively the level of amylase in serum and excretion in urine shows that the amylase lost to the serum after the peak is reached cannot by any

means be accounted for in the urine. It is perfectly apparent that the drop of hundreds of units per cubic centimeter in the blood with a blood volume of 1,500 to 2,000 cc. is not reflected in the urine where the largest change from day to day does not exceed 10 units.

We have often been surprised during the last few years by the stability of the amylase of pancreatic juice and serum kept in the refrigerator. The values will remain constant within the limit of error of the method for weeks particularly in the sterile pancreatic juice. In urine, however, the conditions are not at all the same, very noticeable deterioration occurring in a few hours at room temperature. Pancreatic juice diluted with dog urine 1:1000 gave the expected value if the determination was done immediately but showed a 30 per cent deterioration in 21 hours while a similar dilution of 1:10,000 showed more than 50 per cent loss. Corresponding dilutions with distilled water showed a 60 per cent and 80 per cent loss of enzyme activity.

When tested for deterioration of amylase in shorter periods, urine showed in three samples of 50 to 75 cc. of catheterized urine, representing 2 to 3 hours' renal activity, a nearly immediately noticeable deterioration with 30 per cent to 40 per cent destruction in 6 hours. After that the rate of disappearance became notably slower. Apparently the first few hours while in the bladder at body temperature may have an effect which makes determinations on 24 hour samples entirely misleading, involving an error approaching 50 per cent. However, even allowing for a 90 per cent deterioration of amylase in the 24 hour sample, we cannot account for the rapid changes in serum amylase by excretion through the kidney. Logarithmic extrapolation for zero time of the maximal slopes for deterioration would not raise the values to double the recorded figures. It is very probable that the urine collected for 24 hours can no longer be relied upon to show correct values which would represent the actual amount of amylase which has passed out of the blood in the kidney. A part of the deficit of amylase in the urine is therefore explained by deterioration when diluted in the urine. By far the greater part, however, must be accounted for otherwise.

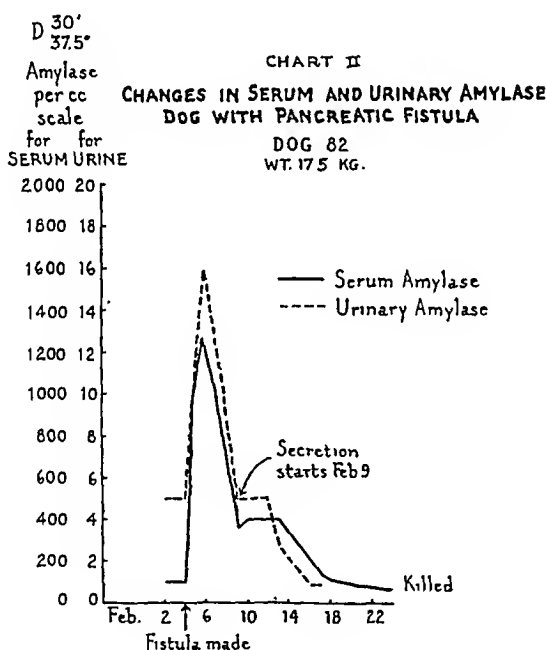
Another mechanism of dealing with high serum enzymes is of considerable interest. This is fully discussed in the early editions of Oppenheimer's *Handbuch der Biochemie* (9, 10). As long ago as 1879, Langendorff (11) suggested that in birds with ligated pancreatic ducts, when the serum enzymes rise, they reach the intestine by some other than the direct route. Abelman (12) and Rosenberg (13) came to the same conclusion. Schegallow (14) determined increased proteolytic activity in the bile after ligation of pancreatic ducts and Lombroso (15) found the same for lipase. Lombroso's observations were verified by Pflüger (16). Our own data indicate that this situation also holds for amylase. In a dog with functioning bile

fistula, the pancreatic ducts were ligated. A comparison of the amylase as well as lipase values before and after the ligation showed that the two enzymes in the bile rose from nearly negligible amounts to a very considerable concentration. This we think is a very important fact and is now the subject of further investigation. It appeals to us as a very ingenious mechanism of returning the pancreatic enzymes to the intestine when temporary occlusion of the ducts occurs. Under these conditions the opinion of Carlson (17) that serum amylase is simply a waste product on the way to excretion has to be somewhat modified. It also offers a satisfactory explanation for the observations made by many observers since the beginning of experimental studies on the pancreas, that the effect on digestion in the intestine without pancreatic juice differs when the pancreas is removed or merely ligated. Cruikshank (18) in a careful study showed that the enzyme digestions in the intestine are really very little interfered with by ligation of the pancreatic ducts while a more serious disturbance ensues when later the whole gland is removed. How much of the digestive load is carried by the enzymes secreted normally by the intestinal mucosa and how much of the disturbance in digestion after pancreatectomy is referable to loss of internal secretion cannot be stated at the present time.

Crandall and Cherry (19) suggest that there is normally a destruction of lipase and to a lesser extent of amylase in the liver. When secondary liver changes set in, due to interference with pancreatic function, the enzyme destroying power of the liver is decreased. A rise in blood lipase then occurs. We do not believe that the rapid fall in blood amylase which we find after the peak has been reached can be explained by any change in rate of enzyme destruction in the liver. Besides, according to Crandall, at the time when the liver changes begin to set in, the enzyme should rise, while as a matter of fact it falls. This subject will be dealt with in more detail further on. Although enzyme destruction in the liver will not explain the drop of amylase after the peak, excretion by the liver may account for it. Curious results are sometimes obtained after ligation of the ducts. It is absolutely necessary to do careful autopsies in all cases. We have encountered both the phenomenon of atrophy—regeneration—reatrophy fully discussed by Bensley (20) and also the establishment of recommunication with the intestine described first by Pawlow (21) in rabbits and by Pratt (22) and co-workers in dogs.

Fistula. When a pancreatic fistula is made the blood and urinary amylase values run a course very similar to that after ligation. This is easily understood because during the first few days after a fistula operation there is virtually a complete obstruction of the ducts. We prefer to wait for the juice to flow spontaneously and do not feed the dogs before operation as Elman does to obtain prompt secretion. The vomiting Elman

records as a constant part of the picture is apparently due to this feeding. The fall after the peak cannot be explained by the establishment of flow because the flow, as seen in chart II, may set in after the beginning of the fall. The mechanism must be similar to that after ligation in which excretion in the bile probably is a large factor. Elman and McCaughan (23) report unchanged values when the blood was taken "several days" after beginning of flow. The initial rise in our curve is to be explained by temporary obstruction and will vary from animal to animal. That given in the chart represents the average result. It begins with the inception of anesthesia. The fall below normal may be due either to lessened resistance to outflow or to functional atrophy. After long-standing fistulas the pan-



creas shows a decreased volume and appears grossly somewhat shrunken without, however, any microscopic cellular changes.

Pancreatectomy. In pancreatectomy a lowering of the serum amylase is recorded by practically all observers but the interpretations of the data vary a good deal. The results are somewhat dependent on the method used. Some of the observations have been made on glycogen instead of starch. There are many indications that the pancreatic polyase acts more specifically on starch, while glycogen is more subject to action of polyases from other tissues. There is some divergence in the results obtained with starch and with glycogen and "a return toward the normal diastase values" after pancreatectomy has been noted only when glycogen was used (24). Willstätter (15) and others also hold that pancreatic amylase hydrolyzes

starch down to maltose only. Serum, however, produces glucose among the starch hydrolysis products. This is interpreted as due to the presence in the serum of maltase which is absent from the pancreas. The presence of maltase, besides leading to divergent results with the various methods for determining extent of hydrolysis, certainly also has an effect through displacement of the equilibrium no matter what method is used. When we try to answer questions regarding the relation of the pancreas to serum amylase all of the above considerations are pertinent. More than elsewhere this is true after removal of the pancreas when we are dealing with the much reduced amylolytic activity of the serum. A résumé of the results on six pancreatectomies is given in table 1. In the first 24 hours there is a drop to somewhat less than half the original value. After this there is no further decided change, the fluctuations being within the error of the method. In a few experiments with glycogen as substrate there

TABLE 1
Pancreas extirpation and serum amylase
Serum amylase D $\frac{30'}{37.5^\circ}$

DOG NUMBER	INITIAL VALUE	DAYS AFTER OPERATION						
		1	2	4	7	18	21	31
86	66.6	26.6	20.0	26.6	26.6			
105	57.2	22.2		22.2				
112	50.0		25.0					
113	80.0	36.3		28.6				
114	66.6		30.8					
118	89.0			40.0		44.5	36.4	35.0

appeared to be a very much smaller initial drop and with some fluctuation a definite tendency to return toward the normal. This coincides with the findings of Milne and Peters (24) and in our experience occurs only when glycogen is used. The starch hydrolysis values never showed a secondary rise even after 31 days under insulin treatment but neither does the serum amylase ever disappear entirely. Very small amounts also continue to appear in the urine. The simplest interpretation would be that a little more than half of the serum amylase comes from the pancreas and the rest has its origin in other organs. We are not certain, however, that this is the only explanation possible. Disregarding for the present the question of strict specificity of the polyases, other tissues may store pancreatic amylase and gradually give it up again when the level in the blood is low. We are also giving further attention to the possibility that the circulating amylase is more slowly excreted the lower the level in the serum. As we have pointed out, although the determination of amylase excreted by the

kidney is very unsatisfactory, the amounts excreted in the urine are very small compared with the concentration in the blood. The threshold for excretion into the bile is apparently higher than that for the kidney since urine always contains quantities at least within the range of ordinary methods while normal bile has hardly more than traces. A good deal is to be said for this point of view which leaves open the possibility that (in the dog, at least) the origin of amylase is exclusively to be sought in the pancreas. Final disposition of this question must be deferred until we know whether or not the amylase of the pancreas and the glycogenase occurring in the pancreas and elsewhere are specific enzymes in the strictest sense.

IS AMYLASE REABSORBED FROM THE INTESTINE? Oppenheimer's (1) review states that amylase is reabsorbed, quoting as the only evidence the old experiments of Wasserthal (26), in which excised intestine was suspended as a dialyzing tube containing saliva and after 24 hours amylase was detected in the outside fluid. After 48 hours there was more. This, of course, proves nothing regarding conditions in the living animal. So far we have found no further evidence for reabsorption of amylase in the literature, but in a great many places the tacit assumption that it is reabsorbed. On the other side of the argument we have the well controlled observations of Moeckel and Rost (27) that when pig's serum, which is ten times as high in amylase as dog's serum, is fed to a dog, no increased amylase can be detected in the dog's serum. A number of experiments were performed on animals under conditions more favorable than those of Moeckel and Rost. Juice was introduced either into the stomach or by duodenal sound into the duodenum, or through an incision directly into the duodenum. There was never any sign of absorption of amylase. The serum samples up to 8 hours afterwards did not vary by one tube in the Wohlgemuth test. As an example we give the following: dog 89; July 15, 1931; juice from dog 89 of July 14 used containing 4,000 units per cc. Two hundred fifty cubic centimeters introduced at a rate varying from 0.5 to 1.5 cc. per minute. Infusion occupied 4 hours. Methylene blue added to the juice was absorbed normally. The initial value of serum amylase was 40 and after $1\frac{1}{2}$, 3, $5\frac{1}{2}$, 7 and 8 hours, the value had not changed. The dilutions used indicated that the assigned value of 40 units was not higher than 45 and not lower than 36. This experiment was by direct infusion into the duodenum, the animal having fully recovered from the operation for tube insertion.

On general grounds we believe it highly improbable that enzymes should be absorbed. There is pretty general agreement that enzymes involve a protein-like structure (on composition of amylase, see Sherman and Gettler, 28) and aggregates of this kind would probably not be absorbed in their original form. If enzymes are generally capable of passing the intestinal mucosa unchanged, it ought to be very easily demonstrable with

amylase from pancreatic juice which has at least 50 times the concentration that occurs in serum. A rather convincing case against absorption is also presented by urease. Evidence of urease action within the body is easily obtained when it is introduced parenterally while urease taken by mouth shows no such results. (See Oppenheimer (1).) In fact, animal life would seem to be a much more precarious thing than it really is if enzymes carried in raw food materials could freely pass the intestinal barrier.

THE EFFECT ON SERUM AMYLASE OF DRUGS WHICH AFFECT PANCREATIC FUNCTION. *Ether*. Elsewhere (29) we have shown that the continuous secretion of a pancreas cannulated by the Rous-McMaster technique is inhibited by ether anesthesia. In the fistula animals we noticed a variable susceptibility to ether anesthesia, and also a variable duration of inhibition of flow. In studying the serum amylase, therefore, we made sure of having a sufficiently long anesthesia period and also a long period of observation after recovery from ether. We found that as a rule it takes two hours to show the first effects. This is not always certain, being too near the limits of the method and we interpret the two hour effect only when there is a continued increase found in the later observations. The peak usually comes at 7 to 9 hours.

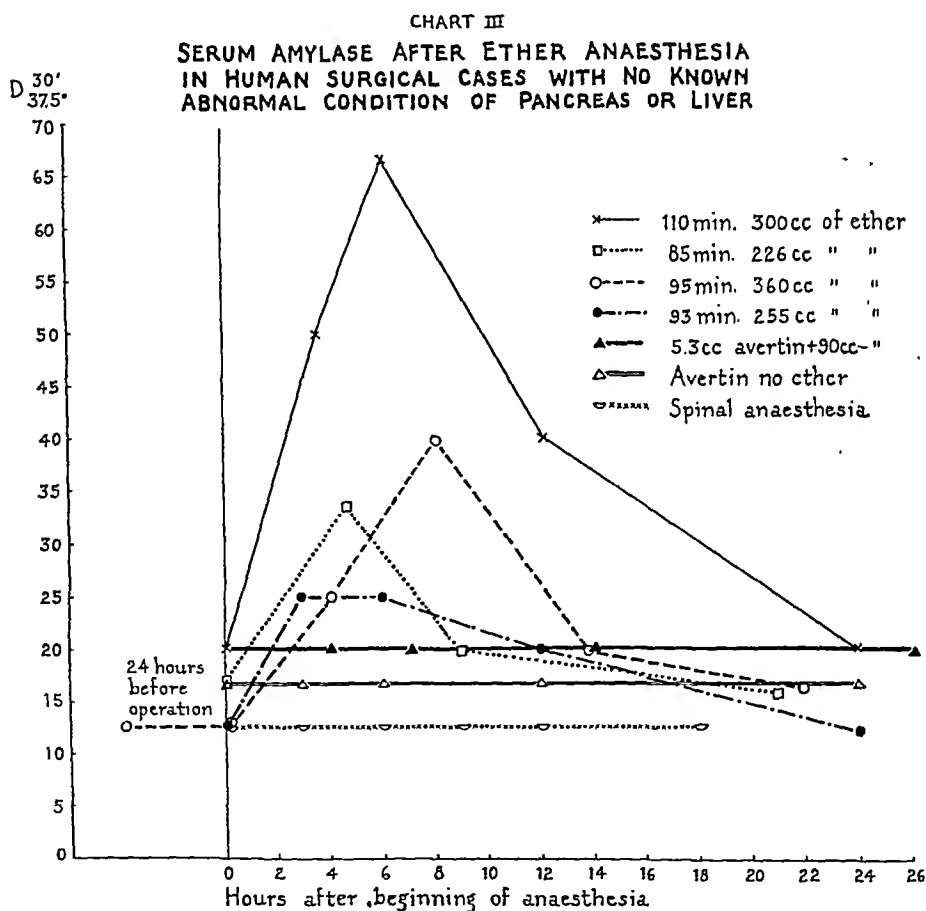
Carlson and Luckhardt (17) and Davis and Ross (30) had made their observations on the effect of ether at maximally two hours, with negative or doubtful results, so that there is no contradiction between their observations and ours. It appears that no matter under what conditions amylase passes from the pancreas into the blood stream about two hours is the minimum time required to show a measurable rise by the methods used. We have never observed an appreciable change in 15 minutes as Elman (31) reports on the basis of a viscosimetric method.

We interpret this rise as being due to an unequal action on the two mechanisms (Anrep) concerned with pancreatic secretion. Ether anesthesia causes an inhibition of the mechanism of juice transfer while the juice (and enzyme) producing mechanism is left unaltered.

There is a close parallelism between mechanical stoppage or clamping of the tube and the ether anesthesia effect. This is shown by the rate at which the serum amylase rises with these various procedures in fasting animals. In four dogs under ether anesthesia the amylase rose at the rate of 20 to 25 units when calculated per hour up to the time of the peak; clamping the rubber tube in a fistula animal resulted in an hourly increment of 20 units, while accidental stoppage (kinks or mucous plugs) gave a rate of 20 to 30 units. A higher rate, of about 50 units per hour, was found only immediately after the operation of ligating the ducts which involves also handling of the gland itself and may certainly be expected to lead to more profound disturbances.

These data leave very little doubt that the effect of ether on blood amylase comes about through an interference with that part of pancreatic function which has to do with the transfer of juice from the cells to the intestine.

In a number of human surgical patients amylase studies after various forms of anesthesia were carried out. The results agree with those on dogs and are given in chart III. Ether produces a marked rise while avertin and spinal anesthesia have no effect.



Chloroform. Before discussing the effect of chloroform poisoning a few words may be in place on the rôle of secondary changes in the liver influencing serum enzymes. Crandall and Cherry (19) claim that in pancreatic lesions or ligation of the pancreatic ducts it is not the condition of this organ that influences serum enzymes, but that the secondary changes in the liver control enzyme level in the blood on the basis of an alleged enzyme destroying power of the liver. We believe with Langendorff, Rosenberg,

Abelmann, Shegalow, Lombroso and Pflüger and from the data in the latest paper of Cherry and Crandall (32) that when the pancreatic function is acutely interfered with and a prompt rise in serum enzymes occurs it is not necessary to invoke secondary changes in the liver which are delayed in their appearance, to explain a rapid rise in serum enzymes. It is certainly true that by the time the marked rise in serum amylase has occurred after ligation of the pancreatic ducts, the liver still shows a perfectly normal appearance in section. The visible changes set in only after 2 to 3 weeks (33). On the other hand, when primary changes occur in the liver such as in chloroform poisoning a marked outpouring of lipase from the injured or destroyed cells into the blood stream has been recorded (34), (35); Crandall and Cherry claim that they can demonstrate the normal

TABLE 2
Serum amylase and lipase after liver injury
By injection of 1.0 cc. chloroform per kgm.

CONDITION	CAT 719		CAT 720		CAT 722		CAT 723	
	Amylase D _{30'} 37.5°	Lipase as cc. N/50 NaOH	Amylase D _{30'} 37.5°	Lipase as cc. N/50 NaOH	Amylase D _{30'} 37.5°	Lipase as cc. N/50 NaOH	Amylase D _{30'} 37.5°	Lipase as cc. N/50 NaOH
Normal.....	57.2	0.45	40.0	0.40	57.2	0.10	50.0	0.05
After CHCl ₃								
1 day.....					28.6	0.65	44.5	0.45
2 days.....	40.0	0.80	22.8	1.00	25.0	1.10	40.0	1.50
4 days.....					36.3	1.10	44.5	1.40
7 days.....					50.0	0.80	50.0	0.40
14 days.....					Dead		50.0	0.30

enzyme destruction in the liver by shunting the portal blood supply past the liver by means of an Eck fistula which results in a raised serum lipase. It is true that the immediate effect of an Eck fistula is largely only a circulatory shunt, but after some time marked degenerative changes set in. Crandall and Cherry's increase in serum lipase is also delayed. We would hesitate to compare the later results of an Eck fistula with the secondary liver changes resulting from interference with pancreatic function as far as effect on serum enzymes is concerned. In the Eck fistula the liver changes are primary. If in this condition the serum enzymes rise at a time when the liver volume has shrunk and marked changes have set in the result is probably quite parallel to chloroform poisoning in the mechanism which produces it. The time of onset is quicker in the more acute chloroform effect.

In contrast to all the above, we wish to show that there is also a direct

effect of chloroform on the pancreas besides its action on the liver. This cannot be confused with the lipase-liver phenomenon because the direction of its effect is opposite.

The effect of chloroform in lowering serum amylase has been previously described by Davis and Ross (30) who further state that this does not occur in animals with extirpated pancreas. What we wish to emphasize particularly at this point is that the effect of chloroform on amylase is through the pancreas and is to be carefully distinguished from the effect on lipase which occurs by an entirely different mechanism involving the liver.

The level of serum amylase can be influenced by a number of factors involving the pancreas. No marked change in serum amylase level has been produced in which the pancreas does not play a dominant rôle. We are inclined to refer all these changes definitely to effects on one of the two mechanisms involved in the external secretory process. Whenever outflow or transfer to the intestine is interfered with the amylase rises. This is brought about by mechanical effects, including trauma or by ether anesthesia. It would be interesting to know whether any other drugs produce similar effects. When there is a lowered elaboration of juice (and enzymes) the serum amylase is lowered. Decreased resistance also seems to have this effect. Lowered serum amylase is brought about by fistula by complete removal of the gland (in partial removal trauma may raise it), by atrophy and by chloroform. When the resistance to outflow is normal secretin has no effect on serum amylase.

COMMENT. When we began our studies on the pancreas a number of years ago, we had the impression that the determination of enzyme activity in serum and in urine was not subject to very much useful interpretation for purposes of pancreas physiology. The many contradictions in the literature discouraged us. As the work progressed, however, we recognized some of the obvious pitfalls which could be avoided. The instability of urinary amylase warned us away from any conclusions based on urine examinations. When we encountered regeneration and reatrophy of the pancreas (20) certain apparent contradictions cleared up. We recognized that in long standing ligations the results can be understood perfectly well, but conclusions must be drawn with great care. The curves for serum amylase plotted against time since operation made it clear that the results of any experimental procedure can be judged correctly only if the time element is considered. The striking effects of ether and of chloroform (observations with other drugs have also been in progress for some time) showed that the picture can be greatly modified by factors which so far have received little consideration. A demonstration of the non-absorption of amylase from the intestine assured us that the serum enzyme situation, in one respect at least, is simpler than had been generally assumed. A clearer knowledge of the relation of spontaneous secretion to the secretin

phenomenon will also further our understanding of the physiology of the pancreas. We believe that on the basis of these and other considerations it is worth while to reinvestigate the whole subject. The progress that has in recent years been made on the chemistry of enzymes warrants the hope that the attempt at biological application of such knowledge may be fruitful. It is our desire in this paper to lay down certain fundamental biological observations which seem necessary for a more intensive study.

SUMMARY. There is little positive evidence that amylase from other organs besides the pancreas can affect the serum amylase. Here it differs from serum lipase which may easily be increased through tissue injury particularly in the liver. Through mechanisms inherent in the pancreas, the serum amylase may be either raised or lowered.

Amylase is not absorbed from the intestine. We believe that, except for excretion into bile or urine, any definite changes in serum amylase level are referable to the pancreas.

The following illustrates the changes so far studied experimentally:

Factors affecting serum amylase

THROUGH THE PANCREAS		NOT THROUGH THE PANCREAS
Raising	Lowering	
Ligation (early stage) Fistula (early stage) Ether anesthesia (Trauma)	Extirpation Fistula (late stage) Ligation (late stage) Chloroform poisoning	Excretion by kidney Excretion in bile

CONCLUSIONS

1. After pancreatectomy the serum amylase falls to less than half the normal value and does not recover. When glycogen is used as substrate the measured effect is less and also inconstant.

2. In animals with ligated pancreatic ducts there is a progressive rise in serum amylase for two or three days. A steep fall to nearly normal values ensues in the next few days after which it slowly sinks to values about half the normal.

3. In fistulas the serum amylase runs a course similar to ligation with, however, a less steep rise and a quicker return to normal.

4. The effect of ether anesthesia is equivalent to a temporary obstruction. This is also shown in human beings.

5. Chloroform lowers the serum amylase with recovery to normal in a few days. It raises the serum lipase (substrate: triglycerides).

6. Amylase is not absorbed from the intestine.

7. Normal liver bile contains only traces of amylase. When the serum amylase rises, excretion in the bile occurs.

8. Amylase is readily excreted in the kidney but there is considerable destruction in the urine. Urine amylase determinations are therefore not very significant.

BIBLIOGRAPHY

- (1) OPPENHEIMER, C. *Fermente und ihre Wirkungen*. Leipzig, 1925, vol. i.
- (2) ANREP, S. V. *Journ. Physiol.*, 1915, xlix, 1; 1, 421.
- (3) FRANCOIS-FRANK ET HALLION, L. *C. R. Soc. Biol.*, 1896, xlviii, 561.
- (4) KOROWITZKY, L. K. *Journ. Physiol.*, 1927, lvii, 215.
- (5) WOHLGEMUTH, J. *Biochem. Zeitschr.*, 1908, ix, 1.
- (6) MICHAELIS, L. AND A. PECHSTEIN. *Biochem. Zeitschr.*, 1914, lix, 77.
- (7) CRANDALL, L. A., JR. AND I. S. CHERRY. *Proc. Soc. Exp. Biol. and Med.*, 1931, xxviii, 570.
- (8) WILLSTÄTTER, R., E. WALDSCHMIDT-LEITZ AND MEMMEN. *Zeitschr. f. Physiol. Chem.*, 1923, cxxv, 93.
- (9) PRYM, O., *Dünndarmverdauung in OPPENHEIMER: Handbuch der Biochem.*, 1909, iii, Part 2, 106.
- (10) ROSENBERG, S. *Pankreas und sein Sekret*, in *OPPENHEIMER: Handbuch der Biochem.*, 1910, iii, Part 1, 122.
- (11) LANGENDORFF, O. *Arch. f. Anat. and Physiol.*, 1879, vii, 95.
- (12) ABELMAN. *Dissert. Dorpat*, 1890. (Reviewed in ROSENBERG (10).)
- (13) ROSENBERG, S. *Pflüger's Arch.*, 1898, lxx, 371.
- (14) SCHEGALOW. *Arch. f. Verdauungskr.*, 1902, viii, 346.
- (15) LOMBROSO, U. *Pflüger's Arch.*, 1906, cxii, 531.
- (16) PFLÜGER, E. F. W. *Pflüger's Arch.*, 1905, cviii, 115.
- (17) CARLSON, A. J. AND A. B. LUCKHARDT. *This Journal*, 1908, xxiii, 148.
- (18) CRUIKSHANK, E. W. H. *Biochem. Journ.*, 1915, ix, 138.
- (19) CRANDALL, L. A., JR. AND I. S. CHERRY. *This Journal*, 1932, xevii, 515.
- (20) BENSLEY, R. R. *Harvey Lectures*, 1915.
- (21) PAWLOW, I. See B. P. BABKIN. *Die äussere Sekretion der Verdauungsdrüsen*. 2 Auflage, Berlin, 1928.
- (22) PRATT, LAWSON AND MARKS. *Trans. Assoc. Amer. Phys.*, 1904, xxiv, 266.
- (23) ELMAN, R. AND McCAUGHAN. *Arch. Int. Med.*, 1927, xl, 58.
- (24) MILNE, L. H. AND H. L. PETERS. *Journ. Med. Res.*, 1912, xxvi, 405.
- (25) WILLSTÄTTER, R. *Untersuchungen über die Enzyme*, Berlin, 1928, i.
- (26) WASSERTHAL, J. *Arch. f. Verdauungskr.*, 1910, xiv, 447.
- (27) MOECKEL, K. AND F. ROST. *Zeitschr. f. Physiol. Chem.*, 1910, lxxvii, 433.
- (28) SHERMAN, H. C. AND A. O. GETTLER. *Journ. Amer. Chem. Soc.*, 1913, xxxv, 1790.
- (29) ZUCKER, T. F., P. G. NEWBURGER AND B. N. BERG. *This Journal* (In press).
- (30) DAVIS, L. H. AND E. L. ROSS. *This Journal*, 1921, lvi, 22.
- (31) ELMAN, R., N. ARNESON AND E. A. GRAHAM. *Arch. Surg.*, 1929, xix, 943.
- (32) CHERRY, I. S. AND L. A. CRANDALL, JR. *This Journal*, 1932, c, 266.
- (33) BERG, B. N. AND T. F. ZUCKER. *Proc. Soc. Exper. Biol. and Med.*, 1931, xxix, 68.
- (34) JOBLING, J. W., A. A. EGGSTEIN AND W. F. PETERSEN. *Journ. Exper. Med.*, 1915, xxii, 707.
- (35) WHIPPLE, G. H. *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 357.

FURTHER OBSERVATIONS ON GLOMERULAR FUNCTION

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In an earlier report (White, 1928) the molecular concentration of glomerular fluid in *Necturus* was stated to be greater than that of serum. The comparisons were made by Barger's technique (1904). Wearn and Richards (1925) had reported a higher concentration of chloride in glomerular fluid than in the serum of frogs. A later paper by Freeman, Livingston and Richards (1930) stated that the percentage of cases in which the chloride content of glomerular fluid was significantly higher than that of the serum in living frogs was lower than in the series of Wearn and Richards. In three other papers simultaneously published from Richards' laboratory it was reported that (Richards and Walker, 1930) the concentration of dye in the glomerular fluid of frogs was the same as in a plasma ultrafiltrate, that (Walker, 1930) the molecular concentration of glomerular fluid in frogs and in *Necturus*, as compared by Barger's method, is the same as that of the plasma, and that (Bayliss and Walker, 1930) the electrical conductances of the glomerular fluid and of a plasma ultrafiltrate were the same. Instances where the glomerular fluid appeared more concentrated than the plasma were thought to be due to experimental errors, the most important of these being evaporation of the glomerular fluid.

Before the publication of the 1930 series of papers Doctor Richards had informed me of the essential findings. I accordingly began in October of 1929 a second series of experiments with the Barger technique, which are reported in this paper. It is believed that the various opportunities for error, among which are the possibilities of evaporation or diffusion through the capsular wall, leakage around the site of the puncture, damage to the glomerular membrane by the mechanics of the puncture or by interference with the glomerular circulation, drawing up of tubular contents in the frog and *Necturus* and in *Necturus* the further possibility of entrance of external fluid through the nephrostome, contamination by the mercury in the pipette and particularly evaporation of glomerular fluid in transferring from collecting pipette to capillary, have been more adequately appreciated and guarded against than in my earlier work. Since the results reported here are essentially a confirmation of results previously reported from Rich-

ards' laboratory, although the experimental procedures have differed somewhat, the details of technique will be omitted.

Apparently the most important defect in my earlier work (1928) was in the technique of transference of fluid from pipette to capillary. The work reported in the present paper consists of 89 experiments on *Necturus* and 6 on the frog and has extended over a period of $2\frac{1}{2}$ years. Suffice it to say that as the degree of protection against evaporation has increased the percentage of cases in which the molecular concentration of glomerular fluid and of serum were the same has increased. My method for preventing evaporation has been to carry out the transfer of fluid inside a moist chamber containing air saturated with water vapor. In a series of 21 experiments on *Necturus* performed after satisfactory saturation had been attained the concentration of glomerular fluid was much greater than that of the serum in 2 cases, slightly greater or with doubtful difference in 5, the same in 15 and less in 1. Contamination by tubular fluid was excluded by maintaining positive intracapsular pressure.

In a series of 5 experiments recently carried out in Doctor Richards' laboratory I collected glomerular fluid from *Necturus* and transferred it to a capillary within a column of paraffin oil. It was then taken over by Doctor Walker and assistants. Their technique for preventing evaporation is to protect the fluid by oil from contact with the air. In these 5 experiments the concentration of fluid was the same as that of the heparinized plasma. In 2 of these cases the concentrations of reducing substances in glomerular fluid and in plasma were determined and found to be essentially the same; this is mentioned as a further indication that the concentration of the fluid had not been changed during or after collection.

In the 6 experiments on the frog I have found the concentrations of glomerular fluid and serum to be the same, although no moist chamber was used. My only explanation of the failure of evaporation to cause trouble here is that smaller tubes, about 400 micra bore, were used in these experiments than with *Necturus*.

Another source of error with *Necturus* which has been described separately (White and Lucas, 1932) is the drawing in of fluid from the outside through the nephrostome. Adequate protective measures are now recognized but a failure to appreciate this danger led Schmitt and White (1928) into error regarding the phosphate concentration of glomerular fluid in *Necturus*. Our finding that the inorganic P content of glomerular fluid was much lower than that of serum was undoubtedly due to our diluting the fluid with phosphate-free salt solution by way of the nephrostome. Walker, Ellinwood and Reisinger found, as they reported at the Philadelphia meeting of the American Society of Biological Chemists, that the inorganic P content of glomerular fluid was the same as that of the plasma in frogs and only a few per cent lower in *Necturi*. These findings were

communicated to me by Doctor Richards and at his invitation I came to Philadelphia and collected glomerular fluid from Necturi, the inorganic phosphate content of the fluid and plasma being determined by Walker and Ellinwood. In 4 fluids, collected with adequate precautions against the entrance of fluid into the capsule from below, the phosphate content was essentially the same as the average of plasma taken immediately before and immediately after the collection, averaging 6 per cent lower. The figures are seen in table 1.

In two cases, 4/21/32 B and 4/22/32, additional collections of glomerular fluid were made from the same capsules but employing a negative

TABLE 1

DATE	GLOMERULAR FLUID	PLASMA		PER CENT DIFFERENCE
		Average	Before After	
	<i>mgm. P per 100 cc.</i>	<i>mgm. P per 100 cc.</i>	<i>mgm. P per 100 cc.</i>	
April 20, 1932.....	5.9	6.2	6.5 5.9	-5
April 20, 1932 B.....	2.6*	3.0	3.0 3.0	-13
April 21, 1932.....	3.8	4.1	3.9 4.3	-7
April 21, 1932 B.....	3.2	3.4	3.2 3.6	-6
April 22, 1932.....	3.2	3.2	2.9 3.4	0
Average.....				-6

* Duplicate.

pressure. A second collection from the capsule designated in table 1 as 4/21/32 B but with a pressure such that capsule wall was just collapsed showed 2.6 mgm. P per 100 cc.; a third collection from the same capsule, employing a greater negative pressure, showed only 1.5 mgm. P per 100 cc. while the plasma before and after collection had 3.6 and 3.8 mgm. P per 100 cc. A second collection from capsule 4/22/32 of table 1, employing a negative pressure several millimeters of mercury greater than required just to collapse the capsular wall, showed 1.3 mgm. P per 100 cc. while the plasma had 3.4 mgm. per 100 cc. In these collections where the leveling bulb was deliberately lowered so that a positive intracapsular pressure was no longer maintained, thus reproducing as far as was possible the

conditions of the earlier work of Schmitt and myself, the phosphate content of the glomerular fluid was much less than that of the plasma, while from the same capsules fluid collected under positive intracapsular pressure had shown essentially the same phosphate content as the plasma. This discrepancy is almost certainly due to the drawing in of phosphate-free solution from the outside when positive intracapsular pressure is not maintained, as is shown by the entrance of dye when the levelling bulb is lowered.

A paper (White, 1929) in which it was shown that a glomerulus may continue to eliminate fluid at a time when the sum of intracapsular pressure and measured plasma colloidal osmotic pressure exceeds the measured glomerular capillary pressure may be briefly referred to. This was interpreted as meaning that the glomerulus may continue to eliminate fluid by some process, presumably secretion, at a time when filtration is no longer possible, although under normal circumstances there is an adequate excess of filtering pressure. Since changes in the permeability of the glomerular membrane cannot be excluded in this work it is recognized that one who does not believe in glomerular secretion could, by making not unreasonable assumptions as to the effective colloidal osmotic pressure across the glomerular membrane under the conditions of the experiments, interpret this work as evidence that glomerular function is a pure filtration process.

It is my opinion the best evidence at present available strengthens the view that glomerular function is a passive filtration process. I am convinced that with present knowledge of the existent dangers and the measures available to circumvent them it is possible to collect normal glomerular fluid from the amphibian kidney. The technique of capsular puncture has long since developed beyond the point where such gross errors as touching the glomerular tuft with the pipette tip, uncertainty of the exact position of the tip within the capsular space, undue trauma of the capsular wall or uncertainty as to the adequacy of a glomerular circulation should occur.

SUMMARY

In a series of 89 experiments on *Necturi* extending over $2\frac{1}{2}$ years the percentage of cases in which the molecular concentration of the glomerular fluid was the same as that of the serum, as compared by Barger's method, was distinctly correlated with the degree of adequacy of the precautions taken to prevent evaporation of fluid, approaching 100 per cent as the technique approached the ideal. Evidence is offered that the earlier findings of Schmitt and White on inorganic P content of glomerular fluid were due to a dilution of fluid from an external source. Glomerular function is very probably a passive filtration process.

BIBLIOGRAPHY

- BARGER, G. 1904. *Journ. Chem. Soc.*, lxxxv, 286.
- BAYLISS, L. E. AND A. M. WALKER. 1930. *Journ. Biol. Chem.*, lxxxvii, 523.
- FREEMAN, B., A. E. LIVINGSTON AND A. N. RICHARDS. 1930. *Journ. Biol. Chem.*, lxxxvii, 467.
- RICHARDS, A. N. AND A. M. WALKER. 1930. *Journ. Biol. Chem.*, lxxxvii, 479.
- SCHMITT, F. O. AND H. L. WHITE. 1928. *This Journal*, lxxxiv, 401.
- WALKER, A. M. 1930. *Journ. Biol. Chem.*, lxxxvii, 499.
- WEARN, J. T. AND A. N. RICHARDS. 1925. *Journ. Biol. Chem.*, lxi, 247.
- WHITE, H. L. 1928. *This Journal*, lxxxv, 191.
1929. *This Journal*, xe, 689.
- WHITE, H. L. AND A. M. LUCAS. 1932. *Journ. Cell. and Comp. Physiol.*, ii, 127.

FURTHER CONSIDERATIONS OF THE PROPERTIES OF THE GONAD-STIMULATING PRINCIPLE OF MARE SERUM

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Previous publications on the gonad-stimulating principle of mare serum have dealt primarily with its effect upon the immature female rat and upon its chemical properties.¹ In this paper particular attention will be given to the effect upon the male. In addition, further data will be presented on the effect upon the immature female and on the stability of the substance.

EFFECT UPON MALE RATS.² *Immature males.* Smith and Engle (1927) by means of pituitary implants were able to increase the size of the accessory reproductive organs of immature male rats. However, they found very little effect upon the testes. Later it was shown that hypophysectomy resulted in an atrophy not only of the accessory reproductive organs of the male, but also of the testes (Smith, 1930). He also found that a very remarkable regeneration of the testis occurred following hypophyseal implants in these animals. There was a restoration of spermatogenesis and also an increase in the number of interstitial cells following the implants. Moore and Price (1931) were not able to demonstrate any great effect upon spermatogenesis when hypophyses were implanted into immature male rats.

Table 1 gives the weights of the reproductive organs of injected immature males as compared to littermate controls. These rats were autopsied four days after the first injection. It will be seen that the testes are approximately doubled in size as a result of the injection, whereas there is a still greater response of the seminal vesicles and prostate glands. These rats were given 500 to 1500 rat units. The actual amount of serum injected varied from 5 to 15 cc. In the next section dealing with the reaction in the immature female rat we describe our method of standardization of the gonad-stimulating hormone in mare serum.

¹ Papers dealing with this subject have appeared as follows: This Journal, xciii, no. 1; xciv, no. 3; Anat. Rec., xlix, no. 3, and Endocrinol., xv, no. 3.

² A preliminary report upon the effect of the gonad-stimulating hormone of mare serum on male rats was published in the abstracts of papers presented at the annual meeting of the American Dairy Science Association, July, 1931.

In studying the histology of the testes of these injected animals, we found more conspicuous changes in the tubules than in the interstitial cells. The tubules were greatly enlarged, as compared to the controls, and the number of spermatocytes was increased (figs. 1 and 2). There was a slight increase of the interstitial tissue following injection, but never as conspicuous as that shown by Moore and Price (1931). Their periods of treatment were more extended than were ours.

The seminal vesicles of injected animals were sometimes six times as heavy as those of littermate controls. Some of this increase in weight is due to accumulation of secretory products within the gland and this probably explains why the seminal vesicles of immature rats show a more marked response as regards weight than the prostate.

TABLE 1
The effect of the gonad-stimulating hormone of mare serum upon the genitalia of male rats

DESCRIPTION OF RATS USED	RAT UNITS ADMINISTERED	AGE AT AUTOPSY	NUMBER OF RATS USED	AVERAGE WEIGHTS			
				Body	Testes	Seminal vesicles	Prostate
		days		grams	grams	grams	grams
Immature males on normal diet.....	None	26-29	7	55	0.263	0.007	0.053
	500-1,500	26-29	7	55	0.492	0.023	0.124
Mature males on normal diet.....	None	100-131	14	440	3.788	1.104	1.221
	500-1,500	100-131	16	448	3.854	1.403	1.534
Mature males on low-protein diet.....	None	125-320	7	208	2.427	0.219	0.296
	500-1,500	125-320	5	202	2.555	0.614	0.797

Mature males on normal diet. By referring to table 1, it will be observed that the injection of the gonad-stimulating hormone of mare serum did not produce any conspicuous changes in the reproductive organs of mature males. Also, a histological study of the reproductive organs did not reveal outstanding changes. Perhaps a more prolonged treatment would have been more efficacious. Although the results we obtained were not clear-cut, slight changes of the same nature as those occurring in the reproductive organs of the injected immature males did occur. There is a possibility that greater changes were produced in the seminal vesicles and prostate than we were able to measure. Ejaculation and the consequent emptying of the seminal vesicles may have partially masked our results.

We have also observed hyperemia of the gonads of both sexes after

administration of the gonad-stimulating hormone of mare serum. This was conspicuous in the mature males even though the increase in weight of the testes was not marked (fig. 3).

Mature males on low-protein diet. Guilbert and Goss (1932) in their study of the effects of restricted protein intake on the oestrous cycle and

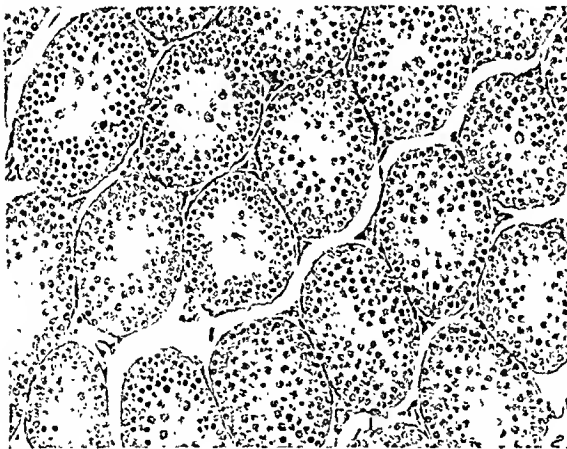
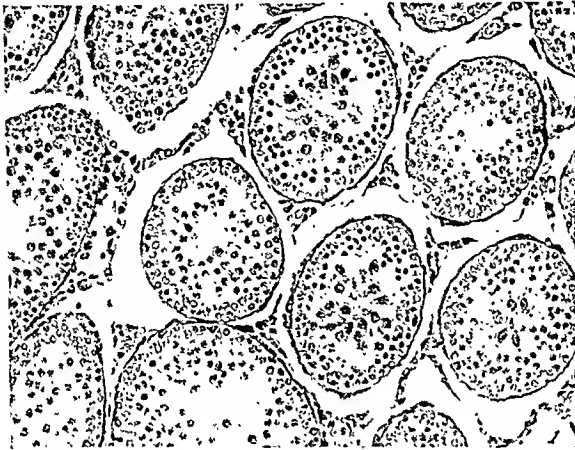


Fig. 1. Photomicrograph of testis of rat B 2688 injected with a single dose of 500 R. U. of the gonad-stimulating hormone of mare serum and autopsied four days later at 29 days of age. $\times 125$.

Fig. 2. Photomicrograph of testis of rat B 2690, littermate control for B 2688, autopsied at 29 days of age. $\times 125$.

gestation in rats found that diets containing 3.5 to 5 per cent protein but otherwise adequate resulted either in cessation of oestrus or in long and irregular cycles. It was also found that male rats, after subsisting for some time on one of these low-protein diets refused to mate when placed with normal females which were in oestrus.

These males were allowed to develop on a normal diet until 50 to 60 days of age, at which time they were changed to the low-protein diet. They usually became impotent after two or three months, during which time there was a significant loss in weight. Examination of the epididymii of males which had become impotent revealed motile sperm. This suggested the possibility that failure to breed might be due to atrophy of the accessory reproductive organs.

Blood serum of pregnant mares was injected subcutaneously into 9 of these impotent males. These males had been tested with normal females before injection with the serum. The number of tests preceding the injection varied from 4 to 12 and only one positive mating was obtained. The



Fig. 3. Reproductive organs of male rats autopsied at 130 days of age: a—rat W 2371 on low protein diet; b—rat G 2392 on low protein diet receiving a single dose of 500 R. U. of gonad-stimulating hormone of mare serum; c—rat G 2394 on normal diet; d—rat B 2395 on normal diet receiving 500 R. U. of gonad-stimulating hormone of mare serum.

usual dose of serum was 5 cc. (500 R. U.). Positive matings were obtained from all but one male on the third or fourth day following injection. The effect of the serum apparently continued for some time since as many as 7 positive matings were secured from individuals tested regularly over a period of 15 days. Out of a total of 30 positive matings 15 were fertile, 14 of which resulted in normal young. One male mated four times during a period of 15 days following injection and all of these matings resulted in litters. This individual mated 4 additional times at irregular intervals during a period extending from 22 to 60 days after injection. None of these later matings was fertile. With another male six positive matings were secured during a 12-day period following injection, none of which

proved fertile, the females in most instances coming into oestrus after the normal interval. In most of these cases of infertile matings, a very small number of sperm was found in the vaginal smears. Some of these males were in extremely poor physical condition.

In addition to the study of the sexual activity of these males on the low-protein diet, we also studied the effect of the injection upon the weight (table 1) and the histology of the reproductive organs. The increase in size of the seminal vesicles and prostate was conspicuous in every instance and was comparable to the increase secured in the immature rats. However, changes in the weight of the testes were smaller and somewhat inconsistent. The weight of the seminal vesicles of one animal was seven times that of its littermate control. This increase in weight is partly due to the increased amount of seminal fluid. Figure 3 gives some indication of the response which may be elicited in these low-protein rats.

The fact that blood serum, rich in the gonad-stimulating principle, induced oestrus in females which had ceased ovulating, and induced sexual activity in males which had become impotent, suggested that the interference with the reproductive process in these rats fed protein deficient diets might be related to hypo-function of the hypophysis.

In order to test further the question of whether the reproductive failure of these rats on a low-protein diet involved the hypophysis, we implanted the glands from some of them into immature females and compared the reaction to that obtained from the glands of littermate controls fed on a normal diet (table 2). A vaginal reaction was obtained in all cases. With one exception, the increase in the weight of the ovaries in those females which received glands from the normal males was greatly in excess of that attained by those receiving the glands from the low-protein males. In the instance where the ovarian response from the hypophyses of low protein animals was equal to that of the controls (recipient B 3044), one individual was included among the donors which had been repeatedly injected with potent serum in the course of breeding tests which were conducted some time prior to autopsy. Whether this accounts for the variance from the rather consistent results obtained from the other six tests is a matter of conjecture.

If rat B 3044 be omitted the average weight of the ovaries of the rats receiving the glands from the controls was about $2\frac{1}{2}$ times that of those receiving glands from the low-protein animals. However, the weight of glands implanted from the controls was nearly double that of the low-protein rats. That the weight of the hypophysis decreased in the rats on the low-protein diet is indicated by the fact that the average hypophysis weight of these rats was 5.1 mgm., while those from 8 normal males autopsied at the same age and weight attained by the low-protein group before being placed on the restricted diet was 8.3 mgm. The weight of hypophysis

per 100 mm. of length (body plus tail) for the low-protein rats was 1.28 mgm. while that for normally-fed males averaged about 2.0 mgm. per 100 mm. of body-tail length.

That the hypophysis is not permanently injured has been indicated by the rapidity with which the normal oestrous cycle is resumed when females on low-protein diets are given adequate nutrition. In some preliminary attempts to determine the amount of gonad-stimulating hormone in the hypophyses of low-protein animals we ground them in saline and injected them into immature females. The response was much poorer than that of

TABLE 2
Implantation of hypophyses of male rats on low-protein and normal diets into immature females

Five glands were implanted into each recipient

DESIGNATION OF RECIPIENT	WEIGHT OF GLANDS IMPLANTED	WEIGHT OF OVARIES OF RECIPIENT*	AVERAGE BODY WEIGHT OF DONORS	AVERAGE LENGTH OF DONORS	DIET OF DONORS
	mgm.	mgm.	grams	cm.	
B3060	27.6	20	202	40.3	Low protein
B3061	27.0	18	230	40.3	
B3054	23.3	18	201	39.4	
B3055	30.0	15	234	41.3	
B3040	21.2	30	204	39.0	
B3044	24.2	79	206	39.8	
G3048	27.2	20	217	40.3	
W3057	39.0	60	406	44.2	Normal
W3058	44.3	70	508	45.6	
W3059	41.3	60	495	45.9	
GH3039	53.3	80	509	45.5	
B3056	49.0	80	503	46.7	
B3042	51.0	78	468	45.5	
GH3045	51.0	60	473	45.8	

* All of the recipients showed a vaginal smear of oestrus on day of autopsy.

the implant method, indicating that it is necessary to use the intact gland to receive the maximal response. We do not feel that the amount of gonad-stimulating hormone present in the gland gives a true indication of its activity. There is some question as to how our results should be interpreted. Perhaps the difference in weight between the hypophyses of the low-protein rats and their littermate controls is more significant than the difference in the reactions of the glands when implanted into immature females.

EFFECT UPON IMMATURE FEMALE RATS. *The assay of the gonad-stimulating hormone of mare serum.* We have found the immature female rat

to be the most convenient test animal for the assay of the gonad-stimulating hormone in mare serum. Before discussing the factors influencing the reaction we will present our method of assaying the hormone. We use rats 25 days old on the day of injection and autopsy them five days later at 30 days of age. If one has no data on the concentration of the hormone it will be necessary to run a preliminary test of the serum to determine the

TABLE 3

The assay of the gonad-stimulating hormone of mare serum

The rats were 25 days old when injected and were autopsied 5 days later at 30 days of age.

DESIGNATION OF RAT	DOSE ADMINISTERED	WEIGHT OF OVARIES	NUMBER OF MATURE FOLLICLES OR CORPORA	VAGINAL REACTION	EXPRESSION OF DOSE IN RAT UNITS
	cc.	mgm.			
W3352	0.01	17	None	—	0.5
G3362		14	None	+	
GH3368		17	None	—	
W564a		14	None	—	
W3374		20	None	—	
GH3375		26	8	+	
Average		18.0	1.3		
G3353	0.02	22	6	+	1.0
G3363		22	5	+	
GH3369		22	7	+	
W565a		12	None	+	
BH3376		20	6	+	
BH3377		22	8	+	
Average		20	5.3		
B3355	0.04	24	6	+	2.0
G3364		27	8	+	
G3370		40	7	+	
B566a		20	8	+	
B567a		21	8	+	
G3378		33	8	+	
Average		27.5	7.5		

approximate concentration. In general it is only practicable to use serum in which the hormone is highly concentrated and, therefore, if 0.04 cc. of the serum does not produce a reaction we discard it. If, however, 0.04 cc. or less will produce the reaction, one can usually determine with fair accuracy the concentration of the serum by injecting 4 groups of rats, consisting of six rats to a group, with 0.005, 0.01, 0.02 and 0.04 cc. respec-

tively. The result of such a test is shown in table 3. The group receiving 0.005 cc. was omitted from the table since all the rats in the group were negative. Our definition of a rat unit is as follows: *A rat unit of the gonad-stimulating hormone of mare serum is the amount which will produce, in a group of six rats, an average of from three to ten mature follicles or corpora for each immature female rat tested and autopsied five days after the injection and half of which amount will fail to consistently produce a vaginal smear of oestrus in another group of six rats.* We will give our reasons for so defining the rat unit of the hormone in question. To begin with, we feel that the vaginal smear should be considered in defining a rat unit as it is at present the most sensitive means for detecting small amounts of the hormone. In the test previously referred to (table 3) 0.01 cc. produced a vaginal smear of oestrus in G3362 although no change took place in the ovaries which could be detected macroscopically. The same was true for W565a receiving 0.02 cc. Thus, if one-half the amount necessary to produce an average of from three to ten corpora or mature follicles fails to produce a vaginal smear of oestrus in all of six rats, one is assured that the end point is being approached at which ovarian changes will be produced. As the vaginal smear changes are determined by oestrin it is evident that the ovary of the immature rat must also be considered. When lower levels of the hormone are given the change in ovary weight as compared to control ovaries is small. For example, the average weight of the two ovaries in a group of 18 control rats autopsied at 30 days of age was 17 mgm. The average weight of the two ovaries in the group receiving 0.02 cc. was 20 mgm. (table 3). Thus it is evident that large numbers would be necessary if weight were used in place of number of mature follicles or corpora.

Factors affecting the reaction. In an effort to determine the factors affecting the character of the gonad-stimulating hormone reaction, we have compared the reaction produced by repeated as compared to single dosages, by various sized dosages, and by rats of varying ages. Table 4 gives the results obtained by repeated as compared to single dosages in 25-day old rats as measured by ovarian weight. It will be noted that the minimal amount which will produce sexual maturity when given in single doses is insufficient to do so when distributed over a 4-day period. Larger amounts given in single or in 8 doses produced similar reactions.

Sixty-four rats were used to compare the reaction in the ovaries of rats 21, 25 and 28 days old at the time of injection. These rats were autopsied 6 days later in order to allow sufficient time to elapse for ovulation to take place. Since this experiment was completed we have found that rats may be autopsied on the fifth day following the injection, that is, if they have not ovulated on the fifth day ovulation will not take place until a later heat period. No significant differences dependent upon age were observed

in the size of the dose necessary to bring on sexual maturity. The ovaries of the older rats were larger than those of the younger rats receiving similar amounts of hormone, but the character of the reaction was very much the same irrespective of age.

TABLE 4

The effect of repeated dosage as compared to single dosage of the gonad-stimulating hormone upon the weight of the ovaries of immature rats

Three rats were used in each group. The rats were 25-days old at the time of the first injection and were autopsied at the 31st day of age

NUMBER OF RAT UNITS ADMINISTERED	AVERAGE WEIGHT OF BOTH OVARIES OF RATS RECEIVING 8 DOSES IN 4 DAYS	AVERAGE WEIGHT OF BOTH OVARIES OF RATS RECEIVING SINGLE DOSE
	mgm.	mgm.
1	19*	25
5	32	38
10	34	32
50	95	124
100	172	174

* Ovaries of all rats in this group were infantile.

TABLE 5

The number of ovulations resulting from the injection of varying size dosages of the gonad-stimulating hormone into rats 21, 25 and 28 days of age on the day of injection

The rats were autopsied 6 days after the injection.

NUMBER OF RAT UNITS ADMINISTERED	21 DAY OLD RATS		25 DAY OLD RATS		28 DAY OLD RATS	
	Number of rats used	Number of rats ovulating	Number of rats used	Number of rats ovulating	Number of rats used	Number of rats ovulating
1	2	1	3	0	2	1
5	2	1	3	1	3	1
10	3	1	3	0	3	1
50	2	0	3	0	3	0
100	2	0	2	0	2	1
1,000	1	0	3	0	2	0

Table 5 presents the ovulation data for 44 of the 64 rats. With doses of 10 rat units or less, approximately one-third of the rats ovulated. Inasmuch as the number of ovulations in the 21-day old rat group was approximately the same as for the 28-day old rat group it is impossible to say that age, within the limits used in this experiment, is a factor regarding ovulation. The oviducts were sectioned serially and examined for ova. All of the ova found were in a state of degeneration, indicating that ovulation had taken place some time before. Engle (1931) has assembled the data

on the relationship between first oestrus and ovulation in the rat and mouse and states that ovulation is coincident with the first oestrus in less than one-half of the instances. Therefore, the number of ovulations resulting from the injection of the gonad-stimulating principle of mare serum approaches that of the first normal oestrus. This is also comparable to the data presented by Engle (1931) in regard to the number of immature mice ovulating following anterior pituitary implants.

It is difficult to determine from the literature the percentage of animals ovulating following the injection of urine of pregnant women or extracts of the urine. Engle (1929) was unable to produce ovulation in the mouse by injection of urine of pregnant women. Although Zondek (1930) has written voluminously on the reaction of the urine of pregnancy in the immature rat and mouse we can find no data which he gives on this point further than the statement that the injection of "Prolan A" under special conditions will produce ovulation. He does not state what these special conditions are. One can only conclude from the data at hand that the injection of urine of pregnancy rarely produces ovulation in the immature rat or mouse.

In regard to the factors affecting the character of the ovarian reaction following the injection of the gonad-stimulating hormone of mare serum, the only one which is clear-cut is the size of dose. Large doses uniformly bring about the mass production of corpora lutea atretica without ovulation. Ovulation occurs when doses up to ten times the minimal dose are given. The data which we have do not support the view that repeated doses are more effective than single dosage. Further, we were surprised to find that the age of the rat within the limits of our experiment was not a factor in regard to the number of rats ovulating. The number of animals in each group is small and it is possible that larger numbers might show some differences in this regard.³

³ We have some data on the number of rat units necessary to produce an ovarian response in other species. Cole and Miller (unpublished data) produced ovulation in 13 out of 15 ewes injected during anoestrus with 50 to 500 R. U. of the gonad-stimulating hormone of mare serum. Lower doses produced very little, if any, change in the ovaries and higher doses resulted in the production of cystic follicles with only an occasional ovulation. Thirteen non-injected controls did not ovulate. A curious feature of this experiment was that none of these ewes bred although tested repeatedly with rams known to be potent during the breeding season. We now have experiments in progress to determine as to whether this failure to breed was due to the impotency of the rams. We feel that it is unlikely that ovulation was produced in the ewes unaccompanied by heat. It appears from our data that 1000 rat units are sufficient to produce oestrus in young sows. As an example the following experiment is presented: Four sows in a litter of eight were given a single injection of 1000, 2000, 3000, and 6000 rat units respectively. The remaining four sows were left as controls. All four of the injected sows showed evidence of heat on the 4th and 5th day following the injection. Three of them were bred, became pregnant, and two of the three gave birth to litters. The third sow of those which became pregnant died of pneu-

DATA ON THE STABILITY OF THE HORMONE. The rats used in the following experiments were twenty-five days old when injected and were autopsied on the fifth day thereafter at 30 days of age. Ovaries of 18 control non-injected rats at 30 days of age had an average weight of 17 mgm. with extremes of 11 and 21 mgm. The average body weight of this group was 78 grams with extremes of 63 and 90 grams. We now regularly autopsy our rats on the fifth day instead of on the fourth in order to check more accurately the number of rats in which ovulation is produced. Only one injection was made excepting in the instances in which 10 cc. were injected and in those instances 5 cc. daily on two consecutive days were injected. The ovarian weights recorded in the table have reference to the weight of the ovaries with oviducts and bursae removed. In all instances the amount injected is stated in terms of original serum. The serum to be used was tested for potency coincidentally with the test of the treated serum.

Effect of storage at 1°-3°C. One of the important characteristics of the gonad-stimulating hormone found in the serum of pregnant mares is its relative stability. We have stored sera in ice cream cartons for approximately two years during which time so much moisture had been lost that the residue was solid and brittle. Upon diluting this solid residue with Locke's solution we found the hormone still present. For example, 27 grams of this residue were diluted with 100 cc. of Locke's solution and then injected. One-tenth cubic centimeter of this dissolved material produced sexual maturity and ovarian development in immature rats. Large doses (1 cc., 5 cc. and 10 cc.) produced ovarian development beyond that produced by the serum when first drawn. Similar results were obtained from retesting a sample of serum after storage in a sealed flask for 200 days. This, and other data, indicates that although the amount to produce a minimal reaction remains fairly constant or increases slightly after long standing, larger doses of the serum after standing produce larger ovaries than similar size doses of fresh serum. Possibly some inhibiting factor is slowly destroyed upon standing.

Effect of oxidation. The above data indicate that the active principle

monia during the course of pregnancy. The fourth sow, which did not become pregnant, was probably bred. She received the largest dose and from other autopsy data which we have on young sows injected with the gonad-stimulating hormone it is likely that this large dose resulted in the production of a large number of corpora lutea atretica. This experiment is not critical, however, as one of the four controls came into heat five days after the injected sows, was bred, and became pregnant. The three remaining controls did not come into oestrus until several weeks later. Other experiments with sows 120-150 days of age would indicate that doses similar to those used in the above experiment will produce oestrus in four to seven days. In a preliminary experiment six cows were given doses of 5000 to 12,000 rat units. They were killed six days later but, unfortunately, were not tested for oestrus. Three of them were given intravenous and three subcutaneous injections. The ovaries were enlarged in all instances, some of them as large as a medium-sized orange.

is not easily oxidized. However, we have studied this point further. Seventy cubic centimeters of serum were shaken for seven hours at room temperature in a 6 liter round-bottom flask in a continuous current of air. The control serum was allowed to stand in a closed flask at room temperature for an equivalent length of time (table 6). Aeration had very little if any effect upon the hormone during this seven-hour period.

TABLE 6

The stability of the gonad-stimulating hormone of mare serum when subjected to oxidation, action of acid, alkali and pepsin

TREATMENT OF SERUM	AVERAGE WEIGHT OF OVARIES RESULTING FROM INJECTION OF:				
	0.01 cc.	0.02 cc.	0.1 cc.	0.5 cc.	5.0 cc.
	mgm.	mgm.	mgm.	mgm.	mgm.
Untreated (control).....		31	40	159	291
Aerated.....		28	64	125	265
Iodine.....		23	29	82	208
Untreated (control).....		49	50	149	209
Acid {	pH 5.....	27	102	140*	180*
	pH 4.....	34	39	260*	210*
	pH 3.....	60	65	150	264*
	pH 2.....	32†	80	125	262
Untreated (control).....	35		48	136	188
Pepsin.....	17‡	26	26	63	251
Untreated (control).....		24	25	118	190
Alkali {	pH 8.....	22	30	81	186
	pH 9.....	28	32	63	258
	pH 10.....	17	22	39	255

* One rat only used in the test. All other ovarian weights represent the average of 2 rats.

† No large follicle or corpora in the ovaries of either of the rats in this group.

‡ Ovaries appeared infantile in both instances but one showed a reaction in uterus and vagina, indicating that some ovarian development had taken place as it takes 30 cc. of this serum to produce a vaginal reaction in a spayed rat.

Next, we treated serum with iodine. The serum was made N/100 with respect to iodine. Only a portion of the iodine was reduced during the half-hour of exposure preceding injection into immature female rats. This treatment did reduce the potency slightly (table 6). The results demonstrate, nevertheless, the stability of the hormone to mild oxidizing agents.⁴

⁴ We have not studied the effect of bacterial action upon the hormone. When it is desired to keep serum for future use it is our procedure to pass the serum through a Berkefeld filter and store it in sterile containers.

Treatment of serum with acid. In previous experiments results obtained by treating serum with pepsin and trypsin were inconsistent (Goss and Cole, 1931). Therefore, we decided to study the effect of acid separately. Five composites of a single sample were taken; one remained untreated except for dilution, the other four were brought to a pH of 5, 4, 3, and 2 respectively with N/3 HCl. All samples were then incubated overnight at 38°C. After incubation the treated samples were brought to a pH of 7.4 with sodium hydroxide. The results appear in table 6. No apparent effect upon the potency resulted until a pH of 2 was reached. One one-hundredth cubic centimeter of serum at pH 2 failed to produce sexual maturity or ovarian development. Thus it is apparent that the pH at which pepsin works would not be particularly injurious to the hormone.

Treatment with pepsin. Inasmuch as acid failed to destroy the hormone, we reinvestigated the effect of pepsin. One per cent of pepsin powder was added to a sample of serum which had been adjusted to pH 3 with N/3 HCl. The sample was incubated overnight at 38°C. After incubation the pH was adjusted to 7.4 and the serum injected. The pepsin powder was previously tested with egg white and found to be highly active.

As may be seen in table 6 there is some loss of potency (approximately one-half). A portion of the serum treated with pepsin was dialyzed. The hormone remained in the sac with the residue as in the case with untreated serum.

Effect of alkali. In a previous paper (Goss and Cole, 1931) we showed that the feeding of large amounts of the hormone was without effect, indicating either that the hormone was not absorbed or that it was destroyed. As we have proved that acid alone or pepsin working in an acid media does not appreciably destroy the hormone, we next studied the effect of alkali (table 6). Possibly some destruction resulted but it was not marked as is indicated by the fact that there was ovarian development in all groups injected with 0.02 cc.

SUMMARY

Immature male rats injected with the gonad-stimulating hormone of mare serum show marked responses in the seminal vesicles and prostate. Also, the testes are approximately doubled in size. The tubules of the testes are enlarged and there is an increase in the amount of interstitial tissue. Mature males on a normal diet do not show marked changes in the reproductive organs following injection although there is evidence of increased secretory activity in the prostate and seminal vesicles. Males on a protein deficient diet show responses in the prostate and seminal vesicles comparable to immature rats but the response of the testes is less conspicuous. Impotency in these low-protein rats is temporarily cured by the injection of the hormone.

Consideration is given to the factors affecting the reaction in the immature female rat. A method of assay of the hormone using immature female rats is suggested. The hormone will produce ovulation when given in small doses. Larger doses result in the production of large numbers of corpora lutea atretica. Data are appended regarding the reaction of the females of other species to the hormone.

The hormone as it occurs in serum is relatively stable in acid and alkali (pH 2 to pH 10) and is not easily destroyed by oxidation or by pepsin.

BIBLIOGRAPHY

- ENGLE, E. T. 1929. *Journ. Amer. Med. Assoc.*, xciii, 276.
1931. *Endocrinol.*, xv, 405.
GOSS, H. AND H. H. COLE, 1931. *Endocrinol.*, xv, 214.
GUILBERT, H. R. AND H. GOSS. 1932. *Journ. Nutrition*, v, 251.
MOORE, C. R. AND D. PRICE. 1931. *This Journal*, xcix, 197.
SMITH, P. E. AND E. T. ENGLE. 1927. *Amer. Journ. Anat.*, xl, 159.
SMITH, P. E. 1930. *Amer. Journ. Anat.*, xlv, 205.
ZONDEK, B. 1931. *Die Hormone des Ovariums und des Hypophysenvorderlappens.*
p. 137, Julius Springer, Berlin.

ON THE INTERACTION OF OESTRIN AND THE OVARY-STIMULATING PRINCIPLES OF EXTRACTS OF THE URINE OF PREGNANCY

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The problem of the antagonism and interaction of sex hormones has been studied quite extensively during recent years. Experiments proving conclusively that the ovary acts as an inhibitor of the effects of the anterior lobe secretions, as well as studies on the functions of oestrin and the corpus luteum hormone in this inhibition, have been carried out. The problem of direct hormonal interaction, however, has not been studied quantitatively heretofore. This is the subject of our experiments.

Evans and Simpson (4), (5), (6) find that hypophyses of gonadectomized animals are much more potent in their ovary-stimulating power than those of normal animals. Engle (2), (3) and Fluhman and Kulchar (7) report identical findings. Meyer, Leonard, Hisaw and Martin (12), (13), studied the effects of continuous injections of oestrin on the stimulating power of the hypophysis of normal and of castrated rats. With the normals they find that the ovaries of recipients of hypophyses from injected animals weigh 40 per cent less than those receiving implants from controls, and with castrates that the ovaries of recipients from injected animals weigh 28 per cent less than ovaries of recipients from castrate controls. They also tested the effect of continuous injection of oestrin into immature rats and find that ovaries of injected animals weigh 40 per cent less than those of controls. Golding and Ramirez (8) report similar results. Kunde, D'Amour, Spencer, Gustavson, and Carlson (10), (11), (16) studied the effect of continuous injection of oestrin on the various organs of the mouse. They find 1, that ovaries of the injected animals weigh 43 per cent as much as those of controls, and 2, that the development of the ovaries is much less in injected animals; no developing follicles are found; 3, a smaller anterior lobe is noted, and hyperplasia of the thyroid.

Doisy, Curtis and Collier (1) studied continuous injections of oestrin, and injection of a massive dose, into immature rats, finding that those receiving a massive dose are always about 10 days behind the normals in weight and development of the generative organs; and that the total growth

of the ovaries of those receiving continuous injections is $\frac{1}{16}$ that of the normal growth at the end of four weeks.

These experiments show a depressive action of the ovary on the hypophysis, and point to oestrin as the depressing agent. Moore (14), (15) demonstrates a similar effect of oestrin on the male hypophysis.

One of the points remaining to be cleared up is the question whether there is direct interaction of the hypophyseal hormones and of oestrin. This point is studied in experiment I by making simultaneous injections of oestrin and the hypophysis hormones into ovariectomized mice.

Mice of the Bagg albino strain, inbred since 1913, were used between the ages of $1\frac{1}{2}$ to 3 months. Absolute uniformity of test animals was assured, as the remotest relationship is that of second cousin.

These mice were ovariectomized in sets of 15 to 20 at a time, and the actual experiment was started 15 days after operation. This arbitrary time was so chosen because the oestrous cycle shown by all ovariectomized mice directly after the operation is certainly completed at that time.

The hormones used were follutein,¹ a preparation of the hypophyseal hormones obtained by extraction from the urine of pregnant women both the luteinizing and the oestrogenic principles, the latter according to our tests being present in excess, and theelin² (oestrin). In one experiment Antuitrin S, a Parke-Davis preparation containing an excess of the luteinizing principle, was used.

The procedure was as follows. Each set of animals was again divided into two sets, 8 to 10 animals per set. These animals were injected on the fifteenth day after operation with simultaneous injections of varying doses of hypophysis hormone and of oestrin. Injections were made dorsally, subcutaneously, and the two hormones were injected in different portions of the back so that no mingling of the hormones could take place under the skin. The hormone preparations were made up by diluting standard preparations with sterile Ringer-Locke solution to the correct concentration, so that $\frac{1}{16}$ cc. of the diluted substance equaled the dosage which the animal was to receive.

If one of these two sets of animals received, for example, an injection of 3 mouse units (m. u.) of follutein, to 1 m. u. of oestrin, the other received 6 m. u.:1 m. u. of oestrin. Vaginal smears were taken from 8 to 12 days. The animals were then all injected with a dose of oestrin equivalent to the one they had received in the first series of injections (in this case, 1 m. u. of oestrin). Vaginal smears were followed again for 8 to 12 days. Then the dosages in the simultaneous injections were reversed, so that the

¹ Through the kindness of Dr. J. F. Anderson of E. R. Squibb & Sons, we received a regular supply of assayed fresh hormone.

² We are indebted to Dr. E. A. Sharp of Parke, Davis & Co. for the Parke-Davis preparations used in these experiments.

animals receiving 3 m. u. of follutein in the first series, now received 6 m. u., whereas those which had received 6, now got 3 m. u. of follutein, again to 1 m. u. of oestrin. Vaginal smears were continued for about 20 days to detect whether regeneration of ovarian tissue had taken place. Those animals not showing regeneration were then used in other preliminary experiments.

This procedure is of great advantage, in that every test animal is its own check. The vaginal smears obtained after oestrin injection are used as a basis of comparison for those smears obtained after simultaneous injections of the two hormones, and it is easy to see whether inhibition of oestrin action has occurred. (The hypophysis hormone alone, of course, has no effect, since no ovaries are present to be stimulated.) It is apparent that a positive inhibition in one animal, in which the oestrin action alone was quite strong, might not be called an inhibition in another animal which did not respond as well to oestrin injection.

One disadvantage of this technique is that since the entire dosage of one hormone is given in one injection, not all the hormone will be absorbed and a portion is excreted without any effect on the organs. However, this disadvantage is met by the fact that every test animal is injected under the same conditions, and that those fractions of the dosage which are absorbed are relatively comparable.

Vaginal smears were taken at regular hours each day so that the interval between two smears was always 24 ± 2 hours. Thus again all smears were made comparable. The first smear was taken 22 ± 2 hours after injection.

The types of inhibition of oestrin action are classified under three headings—total, partial, slight, with a fourth heading including those animals showing no inhibition. The classification is made on the basis of intensity, duration, and delay in appearance of cornification.

Regeneration of ovarian tissue takes place in a large percentage (51 per cent) of the animals. In the case where in spite of regeneration there is an inhibition, there is no need to exclude the animal from the tables; on the contrary the effect is doubly certain, since the follutein neutralized both the injected oestrin and the small amount of oestrin it caused to be secreted. Where, however, no inhibition is gotten and directly afterwards regeneration is evidenced, the animal may be excluded from our tables, because the neutralization may have taken place, but may have been masked by the effect of the oestrin secreted by the fragments of regenerated ovarian tissue. The criterion of regeneration is a cyclical appearance of cornification beginning 10 days after the last injection.

The following two contingency tables—table 1, in which regeneration was not considered, table 2, in which it was taken into consideration, show the distribution in the various dosages, and in the groups of three headings

of low dosage (1 and 2 m. u. follutein vs. 1 m. u. oestrin); medium (3, 4, 5 and 6 m. u. follutein vs. 1 m. u. oestrin); and high dosage (8 and 10 m. u. follutein vs. 1 m. u. oestrin).

TABLE I
Regeneration not considered

OESTRIN: FOLLUTEIN ↓ DOSAGE	INHIBITION				
	Total	Partial	Slight	None	Total
1:1	0	0	2	14	16
1:2	0	1	1	14	16
1:3	5	2	2	4	13
1:4	6	5	0	4	15
1:5	10	6	7	6	29
1:6	3	2	7	1	13
1:8	4	1	3	7	15
1:10	5	5	1	6	17
	33	22	23	56	134

	INHIBITION				
	Total	Partial	Slight	None	Total
Low (1,2:1) dosages:					
Observed.....	0	1	3	28	32
Expected.....	7.87	5.25	5.49	13.37	
Medium (3,4,5,6:1) dosages:					
Observed.....	24	15	16	15	70
Expected.....	17.24	11.34	12.01	29.25	
High (8,10) dosages:					
Observed.....	9	6	4	13	32
Expected.....	7.87	5.25	5.49	13.37	
Total.....	33	22	23	56	134

The lower figure in each cell represents the mathematical expectation, defined by Pearson as the product of the total of the column with the total of the row, divided by the total number of observations.

Table 1 leads to the following conclusions: 1, low dosages. Significantly less inhibition than the mathematical expectation. Correspondingly more than expected in the "none" column. This shows that 1 and 2 m. u. of follutein are not sufficient to suppress the oestrin action. 2, medium dosages. Significantly more inhibition than the mathematical expectation. Correspondingly less than expected in the fourth column. There is defi-

nite inhibition in this range of dosage. 3, high dosages. Very little deviation from the expected values. Interpreted in comparison to the other two rows, this means, that there is definitely more inhibition than in the low, and less than in the medium dosages.

In table 2, a still more sharp division, with the same tendencies as in the preceding table, is seen. The results are more clear-cut, showing relatively more inhibition in the medium dosages.

TABLE 2
Regeneration considered

OESTRIN: FOLLUTEIN ↓ DOSAGE	INHIBITION				
	Total	Partial	Slight	None	Total
1:1	0	0	2	12	14
1:2	0	1	1	10	12
1:3	5	2	1	1	9
1:4	6	5	0	1	12
1:5	10	6	6	3	25
1:6	3	2	5	0	10
1:8	4	1	3	6	14
1:10	5	5	1	5	16
	33	22	19	38	112

	INHIBITION				
	Total	Partial	Slight	None	Total
Low (1,2:1) dosages.....	0 7.66	1 5.11	3 4.41	22 8.82	26
Medium (3,4,5,6:1) dosages.....	24 16.50	15 11.00	12 9.50	5 19.09	56
High (8,10:1) dosages.....	9 8.84	6 5.89	4 5.09	11 10.18	30
Total.....	33	22	19	38	112

The percentage inhibition, i.e., the percentage of the first three columns of the total number of observations is as follows:

1. Not taking regeneration into consideration.....57.5 per cent
2. Excluding mice showing regeneration from the last two columns.....65.2 per cent
3. Not including low dosages, or sub-threshold values.....81.4 per cent

We can conclude, therefore, that interaction has taken place in the test animals.

If neutralization occurs *in vitro*—in a simple chemical reaction—animals injected with mixtures of the hormones should show more inhibition than those injected separately, since the hormones have been in contact longer. The following experiment indicates that this is probably not the case.

	TOTAL	PARTIAL	SLIGHT	NONE	TOTAL
1 m.u. oestrin <i>plus</i> 4 m.u. follutein.....	2	2	3	3	10

The distribution does not differ radically from that in the foregoing tables.

One m. u. of oestrin is shown to be the maximal dose inhibited by 20 or less m. u. of follutein, in the following experiment.

	TOTAL	PARTIAL	SLIGHT	NONE	TOTAL
8 m. u. follutein: 2 m. u. oestrin.....	0	0	0	6	6
10 m.u. F.: 2 m.u. O	0	0	1	8	9
20 m.u. F.: 2 m.u. O	0	1	0	8	9
	0	1	1	22	24

The reason for this is probably due to the fact that in the case of 2 m. u., the absorption of oestrin is too rapid to permit neutralization. In the following experiment the 2 m. u. are given as follows: 1 m. u. with the follutein, the other 5 to 7 hours later. Had this interval been longer, we would probably have gotten more definite results.

	TOTAL	PARTIAL	SLIGHT	NONE	TOTAL
10 m.u. F.: 1 plus 1 m.u. O. (7 hrs. apart)....	0	2	3	4	9
20 m.u. F.: 1 plus 1 m.u. O (5 hrs. apart)...	0	0	2	7	9
	0	2	5	11	18

Another series of injections was made using antuitrin which contains more luteinizing and less oestrogenic hormone. The results cannot be interpreted, first, because there are too few cases, and second, because the relative proportions of oestrogenic and luteinizing hormones in the two preparations used are not so well established that definite conclusions can be drawn.

	TOTAL	PARTIAL	SLIGHT	NONE	TOTAL
4 m.u. antuitrin: 1 m.u. oestrin.....	2	0	2	5	9

Apparently similar experiments with strongly luteinizing extracts of pregnancy urine have been made by De Jongh and Dingemanse (9). Their results were negative. Simultaneous injections of their extract and small amounts of oestrin into immature rats resulted in the suppression of oestrus manifestations, due apparently to ovarian luteinization. Interaction of oestrin and the urine extracts may have occurred, however.

In a second experiment the effect of simultaneous injections of oestrin and follutein into immature mice was studied. The object was to find the dosage of oestrin which would suppress a dose of follutein known to cause good follicular development and ovulation; or, if that was not the case, to observe by histological methods the effect of oestrin *plus* follutein on the ovary, as compared with the effect of the same dose of follutein.

An assay of follutein revealed that a dosage of 4 m. u. given in one injection was sufficient to cause definite follicular development in 100 hours. In the antagonism injections (20 and 10 m. u. of oestrin:4 m. u. follutein) using 5 and 8 animals respectively, it was noticed that follicular development was just as strong if not stronger than in the assay controls. However, there seemed to be less luteinization than in the controls. It appears that 20 m. u. of oestrin are not sufficient to cause suppression of the effects on the ovary of 4 m. u. of follutein. Unfortunately, the effect of higher dosages of oestrin on the same dose of follutein is not available for discussion.

SUMMARY

1. It was observed that oestrin is directly antagonistic to follutein when both are injected into ovariectomized mice.
2. A similar effect is had when the ovary-stimulating hormone is antuitrin S.
3. No definite suppression was had of Follutein effects on the ovaries of immature mice when 10 or 20 m. u. of oestrin were injected simultaneously with the follutein (4 m. u.).
4. A technique for studying hormonal antagonism and interaction is presented. The general method is as follows: Extirpation of the gland studied. Study of effects of removal. Replacement therapy by injection of hormone of that gland. Then simultaneous injection of other hormones to see which of them suppresses or enhances the replacement effect of the first hormone.

BIBLIOGRAPHY

- (1) DOISY, CURTIS AND COLLIER. 1930. *Proc. Soc. Biol. and Med.*, xxviii, 885.
- (2) ENGLE, E. T. 1929. *This Journal*, lxxxviii, 101.
- (3) ENGLE, E. T. 1931. *Endocrinol.*, xv, 405.
- (4) EVANS AND SIMPSON. 1929. *This Journal*, lxxxix, 371.
- (5) EVANS AND SIMPSON. 1930. *Anat. Rec.*, xlv, 216.

- (6) EVANS AND SIMPSON. 1931. *This Journal*, xcvi, 511.
- (7) FLUHMAN AND KULCHAR. 1930. *Proc. Soc. Exper. Biol. and Med.*, xxviii, 501.
- (8) GOLDING AND RAMIREZ. 1928. *Endocrinol.*, xii, 804.
- (9) DE JONGH, S. E. AND E. DINGEMANSE. 1931. *Arch. Néerland. Physiol.*, xvi, 289.
- (10) KUNDÉ, D'AMOUR, GUSTAVSON AND CARLSON. 1930. *Proc. Soc. Exper. Biol. and Med.*, xxviii, 122.
- (11) KUNDÉ, D'AMOUR, GUSTAVSON AND CARLSON. 1931. *This Journal*, xcvi, 677.
- (12) MEYER, LEONARD, HISAW AND MARTIN. 1931. *Endocrinol.*, xv, 17.
- (13) MEYER, LEONARD, HISAW AND MARTIN. 1930. *Proc. Soc. Exper. Biol. and Med.*, xxvii, 702.
- (14) MOORE. 1930. *Proc. 2nd International Congress for Sex Research*.
- (15) MOORE AND PRICE. 1930. *Proc. Soc. Exper. Biol. and Med.*, xxviii, 38.
- (16) SPENCER, GUSTAVSON AND D'AMOUR. 1930. *Proc. Soc. Exper. Biol. and Med.*, xxviii, 500.

THE EFFECT OF STIMULATION OF THE CERVICAL SYMPATHETIC TRUNK UPON THE ENERGY METABOLISM OF RABBITS

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The experiments to be described in this paper were designed to study the changes in energy metabolism following stimulation of the nerve supply of the thyroid gland. Anatomists agree that the gland receives nerve fibers from the cervical sympathetic trunk, and Nonidez (1931) has recently given evidence that in the dog the upper portion of the nerve is the source of most of the fibers to the gland. The question as to whether these fibers exert only a vasomotor influence on the gland or in addition to this a secretory influence is not settled. Mason, Markowitz and Mann (1930) by means of a plethysmographic study have shown that the cervical sympathetic sends vasoconstrictor fibers to the gland. With regard to a secretory nerve supply the evidence is not nearly so convincing. The results of studies of the histological changes following section of the nerve have been conflicting. Thus Misseroli (1909) found characteristic changes in the granules of the secreting cells and Wiener (1909) found a marked reduction in size as a result of section of the cervical sympathetic trunk. Crawford and Hartley (1925), on the other hand, found no histological changes occurring as a result of either section or stimulation of the cervical sympathetic or the vagus and its branches. Evidence gained from a study of differences in iodine content of the two lobes following stimulation of the cervical sympathetic trunk on one side seems to support the idea of a nervous control of the gland cells (Watts, 1915, and Rahe, Rogers, Fawcett and Beebe, 1914).

Early physiological evidence for a nervous control of thyroid activity was given by Asher and Flack (1911) who gave evidence that stimulation of the laryngeal nerves acted like injection of thyroid extract in that both caused an increase in the effect of the depressor nerve upon blood pressure. These workers found also an increased effect of adrenalin in raising blood pressure under the same conditions. Cannon, Binger and Fitz (1914) found in cats a rise of as much as 125 per cent in the rate of energy metabolism following phrenic-cervical sympathetic anastomosis. These results were interpreted as being due to a continuous stimulation of the gland by

impulses originating in the respiratory center. This experiment failed to yield positive results in the hands of Burget (1917), and Marine, Rogoff and Stewart (1917). Electrical changes occurring in the thyroid as a result of cervical sympathetic stimulation were used as evidence for a nervous control of the gland by Cannon and Cattell (1916). Cannon and Smith (1922) demonstrated a 25 per cent increase in rate of the denervated heart as a result of either massage of the thyroid or stimulation of the cervical sympathetic trunk. Neither anemia of the gland nor stimulation of other nerves gave this effect. They concluded that the rise in heart rate was due to increased liberation of thyroid hormone. Rogoff (1918) collected blood from the thyroid vein of dogs before and after stimulation of the thyroid nerves and applied the tadpole feeding test. His results were negative practically. Hektoen, Carlson and Sehulhof (1927) demonstrated by means of a precipitin test that there was no increase in the amount of thyroglobulin in the thyroid vein blood as a result of cervical sympathetic nerve stimulation, direct thyroid massage, or intravenous injections of adrenalin or pilocarpine.

Since the rate of energy metabolism is a fairly reliable index of the state of activity of the thyroid, and the effects of the thyroid hormone are long lasting, it would seem that experiments in which animals were observed for many days following sympathetic stimulation might offer information in regard to the problem of thyroid innervation.

APPARATUS AND METHOD. A modified Haldane open circuit apparatus was used for all of the metabolism tests. The weighings were made on a large balance sensitive to about 20 mgm. The apparatus and method of computation were those described by Marine (1922).

Forty-eight rabbits have been used in this series of experiments, and about six hundred metabolism tests have been made. In order to establish individual normal rates of metabolism each animal had one to five tests before beginning an experiment. In all cases the animals were taken off feed twelve to twenty hours before each test. The operations were carried out under aseptic precautions, and as a result very few animals had to be discarded because of infection. In some cases very light chloral hydrate-urethane anesthesia was used while in others ether was given during the dissection, and no anesthetic during the stimulation. No apparent variation in results could be traced to difference in method of anesthesia.

The experiments carried out may be divided into four groups. In group 1, twenty-six rabbits were used. The cervical sympathetic trunk was exposed on both sides, cut low in the neck, and the cephalic end stimulated with an interrupted tetanic current over a period varying from one to three hours. In order to be sure that impulses were being carried over the nerve as a result of the stimulation, the rabbits' ears were arranged over a light in such a way that vasoconstriction could be readily observed with each period of stimulation.

In group 2 five rabbits were used. Thyroidectomy was performed and the metabolic rate followed until it reached a low hypothyroid level. Stimulation was then carried out as in group 1.

In group 3 six rabbits were used as controls. The sympathetic trunk was cut low in the neck as in group 1, but no stimulation was applied.

In group 4, five rabbits were used. Stimulation was carried out as in group 1, and immediately after, thyroidectomy was performed.

The forty-two rabbits in the above groups and six others have been used in establishing normal metabolism.

Metabolism tests were made in some cases daily and in others every other day for the first ten days after operation, and following this every two to five days over a period, in many cases as long as forty to eighty days.

RESULTS AND DISCUSSION. The rate of metabolism in normal adult rabbits has been found by previous workers (Pommerenke, Haney and Meek, 1930) to average 2.61 calories per kilo per hour. In the present research, the results of tests on forty-eight normal rabbits averaged 2.62 which is in close agreement with the previous series. The figure 2.62 calories per kilo hour is therefore, I believe, a reasonably accurate one for the average rate of energy metabolism in the rabbit. In certain cases, however, animals will be found which show consistently a normal rate of metabolism which varies considerably from the average figure. Because of this fact the data in the accompanying tables are presented not only in percentage variation from 2.62 but also in average percentage variation from the individual normal rates of metabolism.

In group 1 in which stimulation of the cervical sympathetic was carried out, the average maximal variation above the individual normal rate of metabolism, without regard to time of occurrence, was 35 per cent. Twenty-one animals showed a maximal rise of 20 per cent or over, fourteen a rise of 30 per cent or over, nine a rise of 40 per cent or over, six a rise of 50 per cent or over, and five a rise of 60 per cent or over. In nineteen of the twenty-six animals used this maximum occurred between the second and eighth days following stimulation. Typically the rate began rising on the second day. In twelve of the twenty-one rabbits showing a maximal rise of 20 per cent or over, the metabolic rate was followed until it fell to within 12 per cent of the normal. This occurred in five cases between thirty-one and sixty days; in three between sixteen and thirty days; in one at sixty-five days; and in three at five days or less. The rise in rate in the case of the latter three being so much shorter than the others may reasonably be referred to other sources than the thyroid. The results on the animals in group 1 have been summarized in table 1 with regard to the number of days following stimulation. This obscures the high maximal variations already mentioned, but gives a better view of the experiments as a whole. According to this table the rate of metabolism begins rising significantly

on the second day following stimulation, reaches a maximum at eleven to fifteen days, and falls to within 10 per cent of the normal at forty-one to sixty days. Since the rate of metabolism rises markedly and remains at a high level for a number of days, I believe the evidence is very much in favor of the view that there has been an increased activity of the thyroid due to sympathetic stimulation.

To show that the presence of the thyroid gland is necessary in the production of the rise in metabolism, the cervical sympathetic trunk was stimulated in thyroidectomized animals and the rate of metabolism followed. In five such animals (group 2) which showed an average metabolic rate of 1.71 Cal. per kilo per hour or 35 per cent below the normal figure,

TABLE 1

Metabolism of 26 rabbits following stimulation of the cervical sympathetic trunk (group 1)

DAYS FOLLOWING STIMULATION	NUMBER OF ANIMALS	NUMBER OF TESTS	AVERAGE CALORIES PER KILO PER HOUR	PERCENTAGE VARIATION FROM 2.62*	AVERAGE PERCENTAGE VARIATION FROM INDIVIDUAL NORMAL
				<i>per cent</i>	<i>per cent</i>
1	19	19	2.71	+3.4	+7.4
2	16	16	3.04	+16.0	+19.0
3	20	20	3.17	+21.0	+21.0
4	19	19	2.97	+13.4	+18.0
5	16	16	3.09	+18.0	+22.0
6-10	23	54	3.06	+16.8	+20.0
11-15	15	29	3.27	+24.7	+29.0
16-20	13	21	3.05	+16.7	+23.0
21-30	13	36	3.15	+20.3	+23.0
31-40	12	29	2.97	+13.4	+17.0
41-60	9	38	2.74	+4.3	+9.6
61-90	5	20	2.53	-3.4	+1.6

* Average of 135 tests on 48 normal rabbits.

stimulation of the cervical sympathetic was performed. During the first two days following stimulation the metabolic rate rose to 17.5 per cent above the previous hypothyroid level. On the third day, however, the rate had fallen to within 5 per cent of this level, and during the succeeding days it did not rise above that figure. Table 2 (a) shows the effect of stimulation of the cervical sympathetic trunk in these thyroidectomized animals. The results seem to indicate that the presence of the thyroid gland is essential to the long-lasting rise in metabolic rate which occurs following cervical sympathetic nerve stimulation.

The question of the involvement in our experiments of factors other than the nervous effect on the gland in the production of the rise in metabolic rate has been investigated as follows. Section of the cervical sympathetic

trunk was performed in six animals (group 3) and no stimulation was applied. It was thought that the effect of any increase in blood flow through the gland as a result of removal of the vasoconstrictor nerve supply might thus be determined. The animals in this group showed an average maximal variation above the individual normal, without regard to time of occurrence, of 14 per cent. Four of the six rabbits in this control group never varied more than 15 per cent above their individual normal figures.

TABLE 2

DAYS FOLLOWING (a) STIMULATION (b) CUTTING	NUMBER OF ANIMALS	NUMBER OF TESTS	AVERAGE CAL- ORIES PER KILO PER HOUR	PERCENTAGE VARIATION FROM (a) 1.71 (b) 2.62	AVERAGE PERCENT- AGE VARIATION FROM INDIVIDUAL PREVIOUS RATE
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(a) Metabolism of 5 rabbits which had been rendered hypothyroid by thyroid removal and then subjected to cervical sympathetic stimulation. The average metabolic rate before stimulation was 1.71 calories per kilo per hour (group 2)

				<i>per cent</i>	<i>per cent</i>
1	5	5	1.92	+12.3	+13.8
2	4	4	2.01	+17.5	+18.0
3	4	4	1.80	+5.2	+5.0
4	3	3	1.75	+2.3	+5.0
5	3	3	1.73	+1.2	-1.0
6-10	5	10	1.70	-0.6	0.0
11-15	3	4	1.54	-9.9	+8.0

(b) Metabolism of 6 rabbits in which the sympathetic trunk was cut low in the neck but no stimulation applied (group 3)

1	4	4	2.60	-0.8	+3.0
2	5	5	2.63	-0.4	+2.0
3	3	3	2.69	+2.7	+8.0
4	2	2	2.80	+6.8	+13.0
5	4	4	2.71	+3.4	+8.0
6-10	6	16	2.55	-2.7	0.0
11-15	4	6	2.63	-0.4	+4.0
16-20	6	7	2.65	+1.1	+3.0
21-30	5	9	2.56	-2.3	0.0
31-40	5	7	2.60	-0.8	+1.0

The results of this group are tabulated according to post-operative days in table 2 (b). Since in this control group the average maximal variation above the individual normal was only 14 per cent while in group 1 it was 35 per cent, it is readily seen that any increase in blood flow through the thyroid has not been entirely responsible for the rise in energy metabolism following severance and stimulation of the nerve. Although vasomotor effects are not hereby ruled out, the evidence becomes stronger for a secretory activity of the sympathetic on the thyroid.

A graphic representation of the average variations from individual normal rates of metabolism in groups 1, 2 and 3 is given in figure 1.

The next question investigated was whether the rise in metabolic rate is due to liberation of thyroid hormone at the time of stimulation, or to liberation at a later time from a gland made hyperactive as a result of the stimulation. Plummer and Boothby (1921) showed that the greatest rise in metabolic rate occurred on the eighth day following a single intravenous

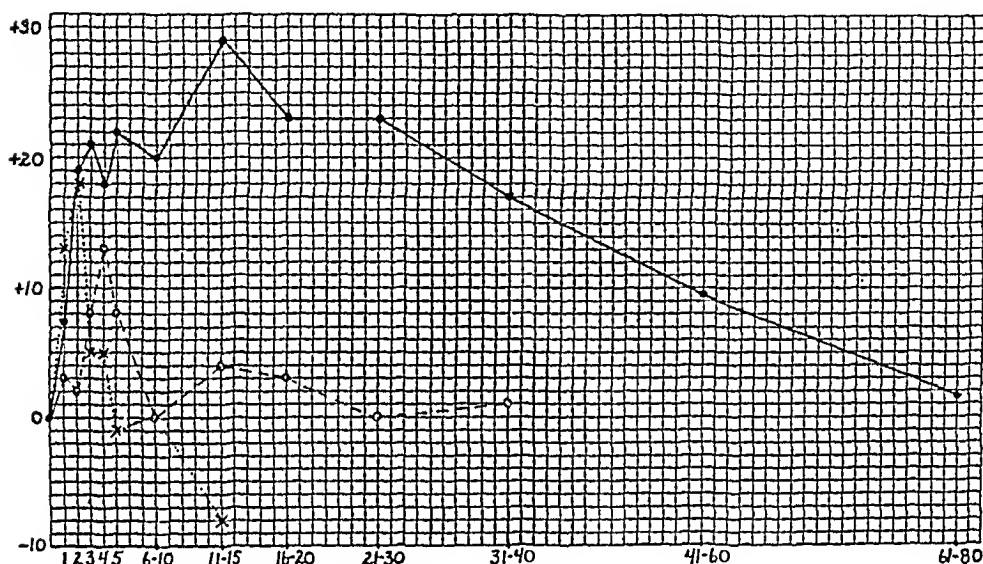


Fig. 1. Abscissae—Days following stimulation.

Ordinates—Average percentage variation from individual normal rates of metabolism except in group 2 as described below.

●—●—The 26 rabbits which were subjected to cervical sympathetic section and stimulation (group 1).

×—×—The 5 rabbits which were subjected to thyroidectomy and allowed to develop a low metabolic rate, and then cervical sympathetic stimulation applied (group 2). The curve represents average percentage variation from the individual hypothyroid levels.

○...○—The 6 rabbits which were subjected to cervical sympathetic section only (group 3).

dose of thyroxin in clinical cases. Later Boothby and Sandiford (1924) showed that the effect may last forty-eight days. These results would seem to indicate that the changes in metabolic rate reported in the present research may be due to liberation of thyroid hormone at the time of nerve stimulation. Kunde (1927), however, showed that as soon as seven to twelve hours following single intravenous injections of thyroxin in dogs, the metabolic rate rose markedly, and then fell to normal within three to five days. Her results would seem to indicate that following sympathetic

stimulation, as in our experiments, a longer-lasting mechanism is at work. In order to present evidence on this point, five rabbits were subjected to sympathetic stimulation and immediately following to thyroidectomy (group 4). The results are tabulated in table 3 (a), and can readily be compared with the results of thyroidectomy alone as given in table 3 (b). It will be seen that even though thyroidectomy immediately followed the

TABLE 3

(a) *Metabolism of 5 rabbits whose cervical sympathetic trunks were stimulated, and thyroid glands removed immediately after (group 4)*

DAYS FOLLOWING STIMULATION AND THYROIDECTOMY	NUMBER OF ANIMALS	NUMBER OF TESTS	AVERAGE CALORIES PER KILO PER HOUR	PERCENTAGE VARIATION FROM 2.62	AVERAGE PERCENTAGE VARIATION FROM INDIVIDUAL NORMAL
				<i>per cent</i>	<i>per cent</i>
1	4	4	3.24	+23.7	+12.0
2	3	3	2.95	+12.6	+7.0
3	5	5	2.61	-0.4	-8.0
4	5	5	2.88	+9.9	+1.0
5	5	5	2.60	-0.8	-8.0
6-10	5	11	2.35	-10.3	-18.0
11-15	5	9	2.31	-11.8	-19.0
16-20	5	7	1.96	-25.2	-31.0

(b) *Metabolism of 9 thyroidectomized rabbits (no stimulation)*

DAYS FOLLOWING THYROIDECTOMY	NUMBER OF ANIMALS	NUMBER OF TESTS	AVERAGE CALORIES PER KILO PER HOUR	PERCENTAGE VARIATION FROM 2.62	AVERAGE PERCENTAGE VARIATION FROM INDIVIDUAL NORMAL
				<i>per cent</i>	<i>per cent</i>
1	4	4	2.56	-2.5	+2.0
2	3	3	2.43	-7.2	+3.0
3	2	2	2.30	-12.0	-14.0
4	5	5	2.41	-8.0	-1.0
5	7	7	2.14	-18.3	-10.0
6-10	6	13	1.93	-26.3	-19.0
11-15	8	12	1.73	-34.0	-27.0
16-20	8	10	1.81	-30.9	-24.0
21-30	6	12	1.72	-34.3	-27.0
31-40	3	5	1.88	-28.2	-20.0
41-60	1	3	1.84	-29.7	-29.0

nerve stimulation, the average rate of metabolism during the first two days is distinctly higher than in the case of the animals thyroidectomized without any previous nerve stimulation. It would seem, therefore, that at least a portion of the rise in metabolic rate may be referred to liberation of the hormone at the time of nerve stimulation. As early as the fifth day following operation, however, those animals which were stimulated before

thyroidectomy showed as low an average percentage variation from the individual normal rate of metabolism as those thyroidectomized without previous stimulation. On the other hand, the animals in group 1 gave an average rate on the fifth day after stimulation of 22 per cent above the normal. These facts indicate that the high metabolic rate found in the latter group is not entirely due to increased liberation of hormone at the time of stimulation, but is due partly to an over-active gland which was brought up to increased activity as a result of the nerve stimulation. The logical conclusion is that stimulation does exert a secretory effect upon the gland cells, this effect being manifest for a considerable time following stimulation.

CONCLUSIONS

1. Stimulation of the cervical sympathetic trunk in twenty-six rabbits resulted in a marked rise in the rate of energy metabolism beginning at the second day, reaching a maximum of 29 per cent above normal at eleven to fifteen days, and returning to normal in forty-one to sixty days.

2. The thyroid gland is essential to the above effects for, in thyroidectomized rabbits subjected to cervical sympathetic stimulation, the rate of energy metabolism does not rise significantly above the hypothyroid level.

3. The increased blood flow through the gland which may be assumed to occur as a result of the cutting of the vasoconstrictor nerve fibers in the cervical sympathetic trunk, does not seem to be the cause of the increase in rate of energy metabolism. Six rabbits failed to show a significant rise following cutting of the nerve, without subsequent stimulation.

4. At least a portion of the increased liberation of thyroid hormone may occur at the time of stimulation. In five rabbits in which thyroidectomy immediately followed sympathetic stimulation, a significant rise in energy metabolism occurred before the fall to thyroidectomy level.

5. Following thyroidectomy of rabbits the energy metabolism begins falling on the fifth post-operative day and continues to fall to an average level of around 30 per cent below normal which is reached in about two weeks.

6. The evidence from our experiments indicates that stimulation of the cervical sympathetic does result in a secretory effect upon the thyroid gland. The rate of energy metabolism in rabbits subjected to this stimulation is 22 per cent above the normal at five days, while the rate in animals subjected to thyroidectomy immediately after stimulation is at the same level at five days as those animals thyroidectomized without any previous stimulation.

BIBLIOGRAPHY

- ASHER, L. AND M. FLACK. 1911. *Zeitschr. f. Biol.*, iv, 83.
- BOOTHBY, W. M. AND I. SANDIFORN. 1924. *Physiol. Rev.*, iv, 69.
- BURGET, G. E. 1917. *This Journal*, xlv, 492.
- CANNON, W. B., C. A. L. BINGER AND R. FITZ. 1914. *This Journal*, xxxvi, 363.
- CANNON, W. B. AND McK. CATTELL. 1916. *This Journal*, xli, 58.
- CANNON, W. B. AND P. E. SMITH. 1922. *This Journal*, lx, 476.
- CRAWFORD, J. H. AND J. N. J. HARTLEY. 1925. *Journ. Exper. Med.*, xlii, 179.
- HEKTOEN, L., A. J. CARLSON AND R. SCHULHOF. 1927. *This Journal*, lxxxi, 661.
- KUNDE, M. M. 1927. *This Journal*, lxxxii, 195.
- MARINE, D., J. M. ROGOFF AND G. N. STEWART. 1917-18. *This Journal*, xlv, 268.
- MASON, J. B., J. MARKOWITZ AND F. C. MANN. 1930. *This Journal*, xciv, 125.
- MISSEROLI, A. 1908-1909. *Arch. d. Fisiol.*, vi, 582.
- NONIDEZ, J. F. 1931. *Amer. Journ. Anat.*, xlviii, 299.
- POMMERENKE, W. T., H. F. HANEY AND W. J. MEEK. 1930. *This Journal*, xciii, 249.
- PLUMMER, H. S. AND W. M. BOOTHBY. 1921. *This Journal*, lv, 295.
- RAHE, J. M., J. ROGERS, G. G. FAWCETT AND S. P. BEEBE. 1914. *This Journal*, xxxiv, 72.
- ROGOFF, J. M. 1918. *Journ. Pharm. Exper. Therap.*, xii, 193.
- WATTS, C. F. 1915. *This Journal*, xxxviii, 356.
- WIENER, H. 1909. *Arch. f. exper. Path. u. Pharm.*, lxi, 297.

THE EFFECT OF MUSCULAR WORK AND COMPETITION ON GASTRIC ACIDITY

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During the century since Beaumont's classical experiments on Alexis St. Martin but few observations on man have been added to those from which he drew his inference that moderate exercise facilitates gastric digestion. In 1846 Combe expressed the dual belief that "rest of body and tranquillity of mind for a short time, both before and after eating, are necessary and conducive to healthy digestion." Cannon's work (1925) demonstrated experimentally that certain emotional states such as unpleasant feelings, anxiety, anger or fear may delay secretion and persist in their effect long after the removal of the exciting condition. In 1928 Campbell, Mitchell and Powell studied a series of normal young men given, before exercise, a test meal of bread, meat and potatoes, or a modification of the Boas meal of oatmeal gruel. The severity of the exercise to which these young men were subjected was roughly measured and consisted usually of running around the laboratory, the distance varying from one to four miles. They concluded that such exercise delayed digestion but lighter exercise, walking, had no inhibitory influence upon gastric secretion. In its application to the field of athletics, interest centers not only on the muscular exertion itself, but also on the emotional stress coincident to competition, as influences possibly modifying gastric secretion. The purpose of this study is to add to the data concerning the influence of exercise upon the gastric secretion of man, and to observe the effect upon gastric function of muscular exertion accompanied by emotional excitement.

RESTING GASTRIC ACIDITY. The first series of observations was made on M. M. M., a normal, healthy subject accustomed to vigorous exercise, a young woman of 21 with professional training in physical education. Ewald's test-breakfast was administered 14 to 18 hours after the last meal. A Rehfuß tube was swallowed and the stomach was aspirated one hour subsequent to the beginning of the test meal. The filtrate was titrated and examined for free and total acidity. After the subject had been trained to swallow the stomach tube, a series of ten observations was made to establish the normal resting acidity values following the test-breakfast. The results are expressed in terms of the number of cubic centimeters of

decinormal sodium hydroxide necessary for the neutralization of 100 cc. of gastric juice, each cubic centimeter representing one degree of acidity. Total acidity averaged 47.6° and HCl 29° , 60.92 per cent of the total acidity being due to free hydrochloric acid. From a review of records of gastric analysis made at the Mayo Clinic and from a study of data in the literature, Vanzant et al. (1932) selected a series of 3746 cases for the establishment of age group standards of normal gastric acidity. She and her associates found that the modal free acidity for women is approximately 35 units throughout adult life with a normal range of about 90 units, modal total acidity being practically constant at 51° between the ages of 20 and 60. Subject M. M. M.'s normal resting acidity falls within the limits of these standards.

EXERCISE FOLLOWING THE TEST MEAL. The subject came to the laboratory and in place of resting for the hour between the beginning and the aspiration of the test meal, she rode the electrodynamic brake bicycle ergometer. The muscular exertion was at first equivalent to 336.44 kgm.m./min. at an average pedalling rate of 63 revolutions per minute. It was repeated on ten different days, increasing in severity to 489.36 kgm.m./min. with the training of the subject. Immediately after the termination of the exercise the gastric contents were aspirated and analyzed. Total acidity was moderately reduced, averaging 39.4° . The depressing influence was more marked as regards hydrochloric acid. It dropped by 33 per cent to an average value of 19.3° , but 48.98 per cent of the total acidity of the post-exercise gastric juice being due to free HCl. A separate analysis of the first and last five records reveals that notwithstanding an augmentation in the rate of working, the latter demonstrated a less marked average decrease in free and total acidity, and three individual records surpassed the normal standard. The increase in the severity of the work did not balance the effects of training, and as the subject became more proficient in this exercise it had a decreasing inhibitory influence upon gastric acidity, eventually augmenting both free and total acidity to a level above the resting normal. These findings are demonstrated in figure 1.

EXERCISE BEFORE THE TEST MEAL. Four experiments were performed with 60 minutes of physical exertion preceding the administration of the test meal. The rate of working averaged 550 kgm.m./min. at a mean pedalling rate of 68 revolutions per minute. Immediately after the termination of the exercise the meal was eaten and the subject remained quietly seated until the usual period of aspiration. Total and free acidity both increased moderately, reaching 53.25° and 35.75° respectively, 67.13 per cent of the total acidity now being due to free HCl.

A single observation was made after an exhausting bout of work. Before coming to the laboratory the subject spent six hours in vigorous, outdoor exercise. The usual breakfast was eaten but luncheon was substi-

tuted by 500 cc. of special Guernsey milk and the juice of 8 oranges taken in small quantities at short intervals preceding midday. No food was taken for 7 hours prior to the experimental exercise. The subject then did a severe and exhausting piece of work on the ergometer, riding for 60 minutes, the average rate of working being 703.46 kgm.m./min. The test meal was administered after the cessation of exercise and aspirated in one hour. Both total acidity and free hydrochloric acid were markedly sup-

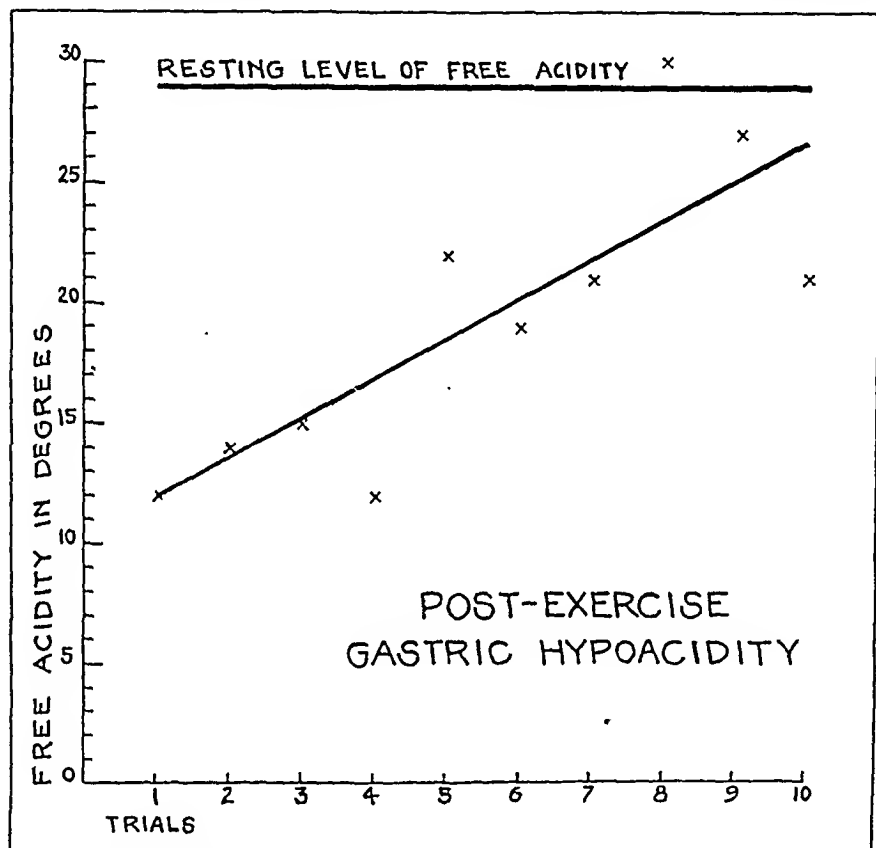


Fig. 1. Ten successive experiments showing the decreasing influence of exercise following a test meal, upon free gastric acidity. Observed HCl in cubic centimeters of N/10 NaOH necessary to neutralize 100 cc. of gastric juice and straight line fitted by least squares.

pressed, occurring in 29° and 6° in the order designated, but 20.68 per cent of the total acidity being due to free HCl.

Figure 2 is a composite of these 25 experiments. It shows that exercise during and immediately following an Ewald meal reduced both total acidity and free acid, figure 1 showing the inhibition to be less marked as the subject became trained. Exercise preceding the test meal augmented gastric acidity, except when it was exhausting, such exercise being followed by a marked diminution in the degree of acidity.

CONFIRMATORY OBSERVATIONS. The gastro-intestinal tract is variable in its response to identical stimuli in different subjects. To confirm the trend of the reactions of a single individual, three different persons were subjected to a series of controlled observations similar to the experimental procedures of M. M. M. These subjects were normal, healthy young women between the ages of 19 and 23, professional students in physical education, above the average in fitness and neuromuscular skill and accustomed to strenuous exertion.

1. *Resting gastric acidity.* The Ewald test-breakfast was administered and aspirated as described. The average of 3 observations was taken as

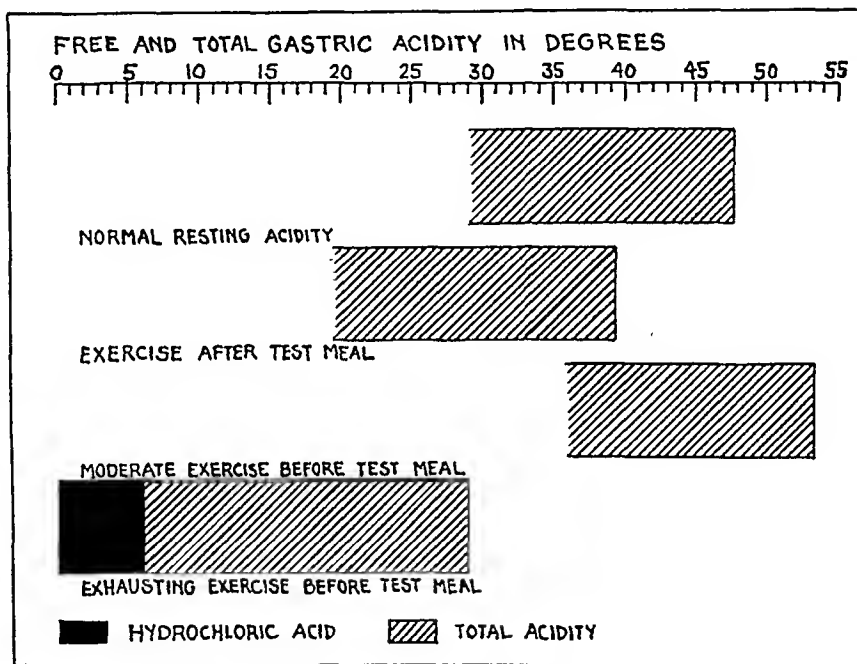


Fig. 2. Bar diagram showing the influence of exercise upon the gastric acidity of one subject.

the normal acidity for each subject. L. D. had an acidity below the modal values set by Vanzant (1932), M. M. closely approximated the standards and S. H. exceeded them. The group thus represents the norm and its extremes.

2. *Exercise following the test meal.* The subjects pedalled the bicycle ergometer for one hour doing a very moderate piece of work without any evidence of fatigue. The influence of the exercise was slight, in general stimulating, average free and total acidity being augmented by 0.4 per cent and 4.2 per cent respectively.

3. *Exercise before the test meal.* The subjects exercised for one hour

immediately before the administration of the test meal. This was the first time they had done so protracted and severe a piece of work on the bicycle ergometer and there were manifestations of fatigue, especially in subjects L. D. and S. H. who showed a post-exercise decrease in gastric acidity. Subject M. M. worked more moderately and the exercise was stimulating. On an average free acidity decreased by 7.1 per cent although there was an increase of 4.2 per cent in total acidity.

The group was subsequently subjected to gentle exercise in the endeavor to determine whether such muscular activity preceding a meal would be uniformly stimulating in the event that the exercise be sufficiently light. The three subjects took a brisk walk out of doors during the hour preceding the administration of the test meal. The results were accordant, on an average free acidity being increased by 25.94 per cent and total acidity by 20.34 per cent.

The attempt was next made to duplicate the severe and exhausting work which had had so profoundly depressing an influence upon the gastric acidity of subject M. M. M. On the day of the experiment the usual breakfast was eaten but luncheon was substituted by highly nutritious liquids. The group took a 28 mile bicycle ride, circumventing a neighboring lake and upon their return to the laboratory performed the usual 60 minute piece of exercise upon the ergostat. Subject S. H. was obviously tired. The bout of exercise was slightly depressing, free acidity decreasing by 12.75 per cent and total acidity by 0.38 per cent. On the other hand L. D. and M. M. showed no evidences of fatigue and the exercise, severe and protracted though it seemed, had a strongly stimulatory effect, free and total acidity augmenting by 81.00 per cent and 66.07 per cent respectively in M. M. and by 56.08 and 82.99 per cent in L. D.

Any exercise of endurance must of necessity be carried on in the steady state. It is known that exercise carried on at high speed incurs an oxygen debt, and the rapid accumulation of acid metabolites results in early exhaustion. The group was finally subjected to a battery of exercises carried on at high speed, the total period of exertion being limited to 5 minutes. The exercise consisted of stair climbing at top speed, rapid exercise on the stationary bicycle, violent rope jumping and rotating a Prony brake ergometer by hand. At the termination of the exercise the subjects were incoordinated, dyspneic and sweating profusely. The test meal was administered at once and aspirated in one hour. A diminution in the degree of acidity occurred in every case, the average reduction being 50.92 per cent in HCl and 33.14 per cent in total acidity.

The individual findings of this series of experiments are recorded in the accompanying tables. In trend, they confirm the observations made on subject M. M. M.

COMPETITION. The influence of competitive sport participation upon

gastric acidity was next studied. The necessity of swallowing a stomach tube for the aspiration of gastric contents imposes from the outset a psychic disturbance which makes it difficult to obtain reliable findings under conditions not too far removed from normal. If the competition is genuine, not artificially imposed, it is difficult to obtain subjects because of

TABLE 1

Showing the average free and total acidity of the three subjects studied

SUBJECT	NORMAL GASTRIC ACIDITY		
	HCl	Total acidity	Per cent HCl
M. M.....	40.33	56.00	72.01
L. D.....	28.83	37.16	64.12
S. H.....	65.33	86.33	75.67

TABLE 2A

Showing the influence of moderate exercise following an Ewald meal upon free and total acidity

SUBJECT	POST-EXERCISE GASTRIC ACIDITY		
	HCl	Total acidity	Per cent HCl
M. M.....	44.00	60.00	73.33
L. D.....	24.00	40.00	60.00
S. H.....	67.00	87.00	77.01

TABLE 2B

Showing the influence of various types of exercise preceding an Ewald meal upon free and total acidity

Post-exercise gastric acidity

SEVERE EXERCISE			GENTLE EXERCISE			ENDURANCE EXERCISE			EXHAUSTING EXERCISE		
HCl	Total acidity	Per cent HCl	HCl	Total acidity	Percent HCl	HCl	Total acidity	Percent HCl	HCl	Total acidity	Percent HCl
49.00	65.00	75.38	53.00	69.00	76.81	73.00	93.00	78.49	29.00	47.00	61.70
25.00	45.00	55.55	44.00	56.00	78.57	45.00	68.00	66.17	9.00	26.00	34.61
51.00	77.00	66.23	73.00	91.00	80.21	57.00	86.00	66.27	28.00	47.00	59.57

the danger of upsetting the "condition" of the players by the necessary modification of the usual pre-game procedure, especially the administration of an unpalatable meal immediately prior to the beginning of play.

The subjects were healthy, normal young women physical education students, participating in the final game of a basketball tournament, one about which many traditions center. On the day of the game the usual

breakfast was eaten. Luncheon was substituted by a high calorie liquid diet of sweetened fruit juice, egg-nog, hot chocolate and malted milk given at hourly intervals during the morning. No food was taken for 7 hours prior to the game, a test meal of dry toast and tea being administered just before the onset of play. Competition was keen and the game was fast and hard fought. At its termination, Rehfuß tubes were swallowed and the gastric contents were aspirated. The subjects had been carefully

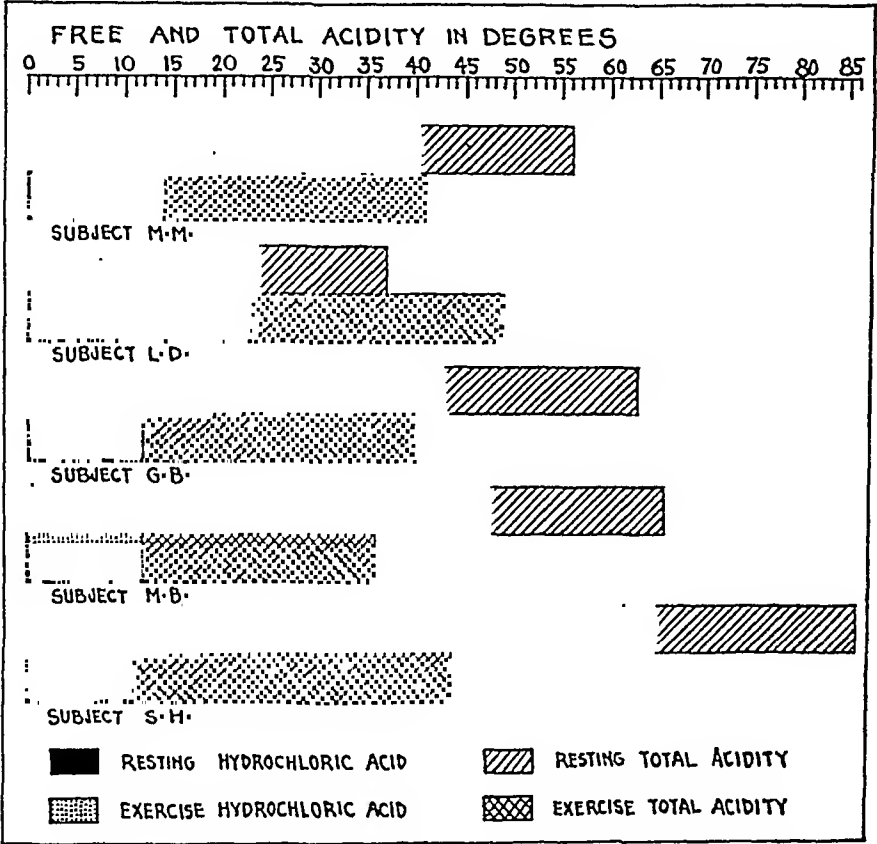


Fig. 3. Bar diagram showing the influence of competitive exercise upon the gastric acidity of five different subjects playing in the same game.

instructed concerning the technique of swallowing the tube and had witnessed the procedure. Five passed Rehfuß tubes without difficulty. The resting and exercise gastric acidities of these subjects are recorded in figure 3. Except for an increase in subject L. D. who played only during the second half of the game, there was a diminution in the total acidity of the gastric secretion. Free acidity was uniformly diminished, in general to a point far below the resting level.

DISCUSSION. In 1927 Apperly and Semmens began to investigate the

relationship of gastric function to the chemistry of the blood. Bennett and Dodds (1921) had already noted that a high alveolar carbon dioxide was associated with high gastric acidity. Apperly with Semmens in 1928 and with Crabtree in 1931 altered the plasma bicarbonate and cH of the blood and showed that the gastric acidity varies directly with the bicarbonate content irrespective of the H ion concentration. During muscular exercise, especially when violent and carried on at high speed, lactic acid is rapidly produced, it accumulates in the blood, lowers the bicarbonate content and accelerates the output of carbon-dioxide. The lungs are unable to wash out the carbonic acid rapidly enough and the H ion concentration rises. In the light of these experimental observations the diminution in gastric acidity during violent exercise may be due to the concomitant fall in plasma bicarbonate. In accord with this hypothesis, hypoacidity was observed to appear only when the muscular exertion was associated with evidences of the incurrence of an oxygen debt and the greatest diminution in gastric acidity occurred when the exercise was severe and exhausting. The physico-chemical changes in the blood must therefore be taken into consideration in the determination of the cause of the exercise gastric hypoacidity.

If the muscular exercise is severe and generalized, splanchnic vasoconstriction diverts blood away from the visceral area to the active skeletal muscles and skin, producing a relative diminution in the oxygen supply to the stomach. This may contribute to the production of the gastric hypoacidity associated with exhaustive muscular exertion. It is common experience that psychic disturbances also modify gastric function. Apperly and Crabtree (1932) stress, in addition, the influence of emptying time upon gastric acidity. No motility observations were made in this series of studies and such may throw further light upon the interpretation of these findings.

Crandall (1928), studying the effect of physical exercise on the gastric secretion of Pavlov dogs, noted that after the cessation of exercise, the secretion may rise to a level above the normal. We observed that when gentle exercise preceded or followed the test meal, gastric acidity might exceed the resting level. Such exercise is usually unassociated with emotional disturbance and neither shunts large quantities of blood away from the visceral area nor induces marked changes in the composition of the blood. Moderate muscular exertion heightens the general metabolism, improves the circulation and has a stimulatory effect upon the organism as a whole, probably thus augmenting gastric functional activity.

SUMMARY

Gentle exercise before or after a test meal augments gastric acidity. Protracted exercise is not necessarily depressing, but exhaustive muscular

exertion, whether it precedes or follows a test meal, is associated with a diminution of the acidity of the gastric secretion to a level below resting normal, and the decrease is greatest when the exercise is accompanied by emotional excitement.

Acknowledgment. Our best thanks are due to the students who generously acted as subjects and withstood the rigors of this research.

BIBLIOGRAPHY

- APPERLY, F. L. AND K. SEMMENS. 1927. Aust. Med. Cong. Supp. to Med. Journ. Aust., ii, 153.
- APPERLY, F. L. AND K. M. SEMMENS. 1928. Med. Journ. Australia, ii, 226.
- APPERLY, F. L. AND M. G. CRABTREE. 1931. Journ. Physiol., lxxiii, 331.
- BENNETT, T. I. AND E. C. DODDS. 1921. Brit. Journ. Exper. Path., ii, 58.
- CAMPBELL, J. M. H., G. O. MITCHELL AND A. T. W. POWELL. 1928. Guy's Hosp. Rept., lxxviii, 279.
- CANNON, W. B. 1925. Bodily changes in pain, hunger, fear and rage. New York, D. Appleton & Co.
- COMBE, A. 1846. The physiology of digestion. New York, William H. Colyer, 199.
- CRANDALL, L. A. 1928. This Journal, lxxxiv, 48.
- VANZANT, F. R., W. C. ALVAREZ, G. B. EUSTERMAN, H. L. DUNN AND J. BERKSON. 1932. Arch. Int. Med., xlix, 345.

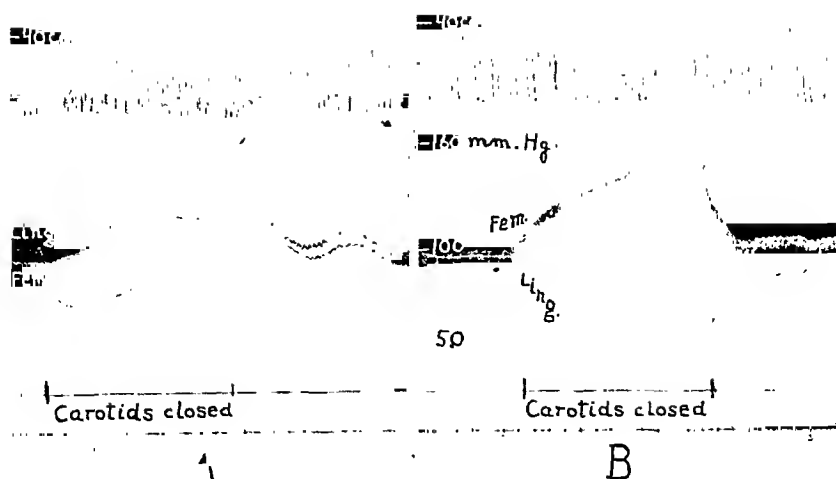


Fig. 3

ERRATA

VOLUME CII, OCTOBER, 1932

Pages 100, 101, 107, 109, 112: Figures 3, 5, 9, 10, 11, and 15 have been reprinted to show greater detail.

Pages 121, 124, 125: Figures 2, 5, and 6 have been reprinted to show greater detail.

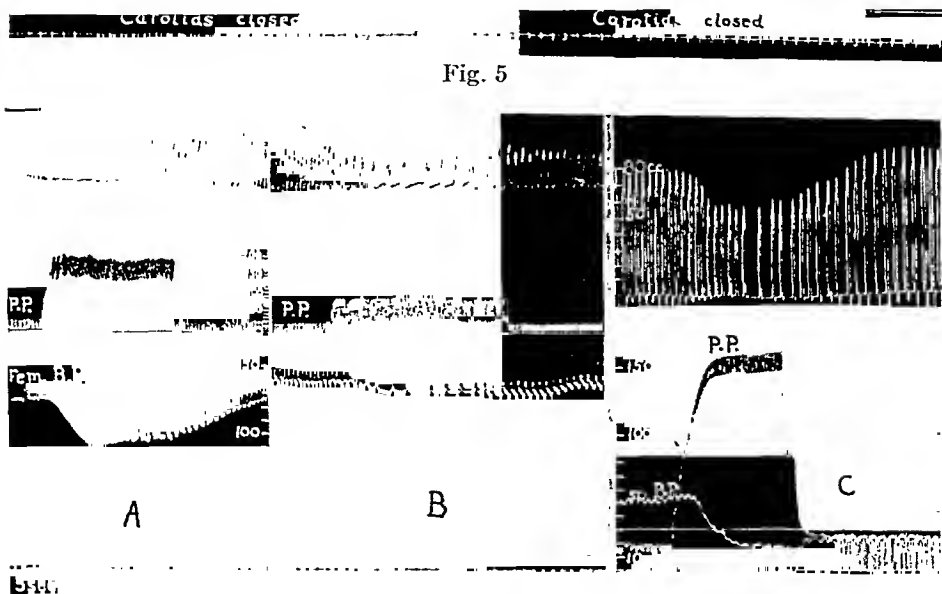


Fig. 9

exertion, whether it precedes or follows a test meal, is associated with a diminution of the acidity of the gastric secretion to a level below resting normal, and the decrease is greatest when the exercise is accompanied by emotional excitement.

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BIBLIOGRAPHY

- APPERLY, F. L. AND K. SEMMENS. 1927. Aust. Med. Cong. Supp. to Med. Journ. Aust., ii, 153.
- APPERLY, F. L. AND K. M. SEMMENS. 1928. Med. Journ. Australia, ii, 226.
- APPERLY, F. L. AND M. G. CRANTREE. 1931. Journ. Physiol., lxxiii, 331.
- BENNETT, T. I. AND E. C. DODDS. 1921. Brit. Journ. Exper. Path., ii, 58.
- CAMPBELL, J. M. H., G. O. MITCHELL AND A. T. W. POWELL. 1928. Guy's Hosp. Rept., lxxviii, 279.
- CANNON, W. B. 1925. Bodily changes in pain, hunger, fear and rage. New York, D. Appleton & Co.
- COMBE, A. 1846. The physiology of digestion. New York, William H. Colyer, 199.
- CRANDALL, L. A. 1928. This Journal, lxxxiv, 48.
- VANZANT, F. R., W. C. ALVAREZ, G. B. EUSTERMAN, H. L. DUNN AND J. REEFSON

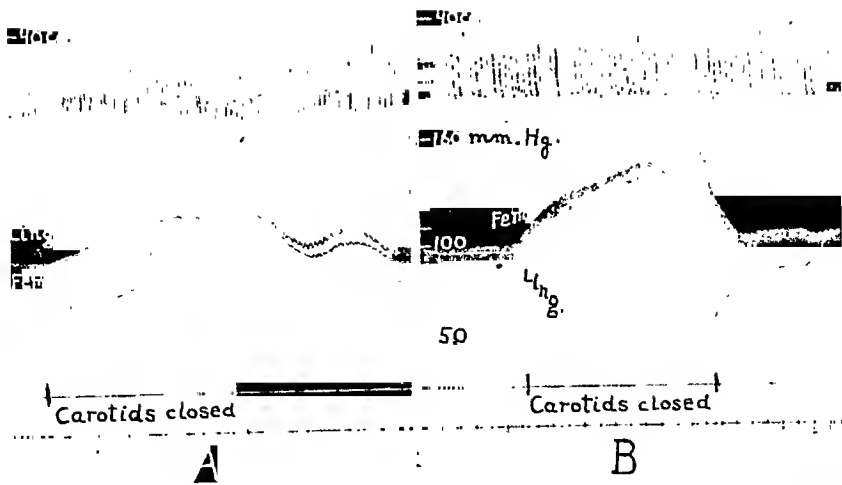


Fig. 3

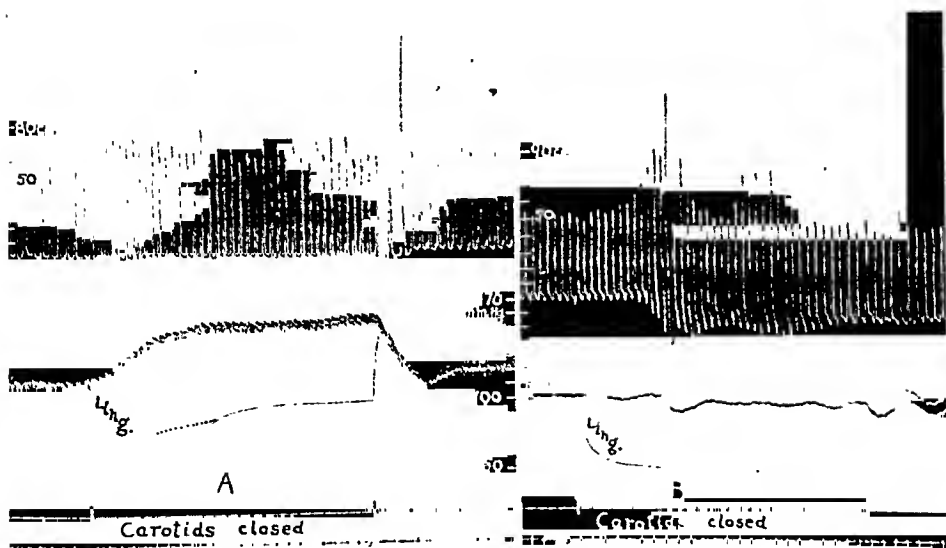


Fig. 5

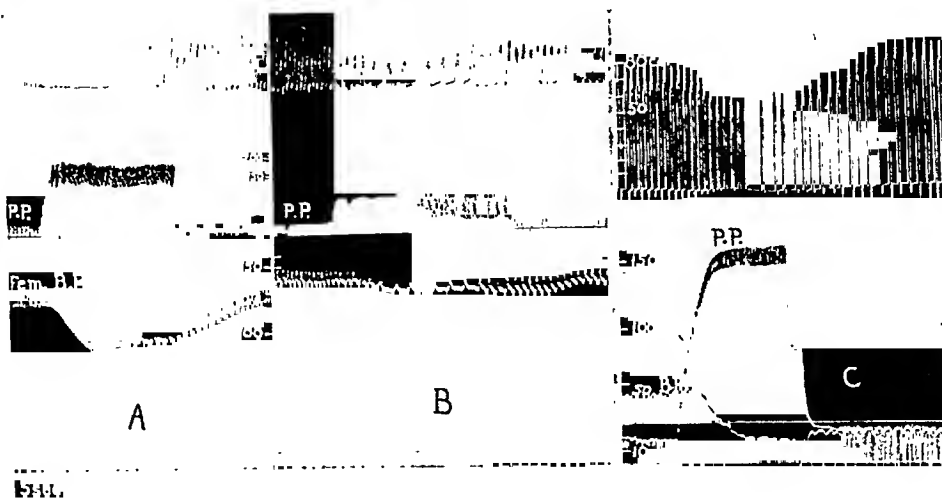


Fig. 9

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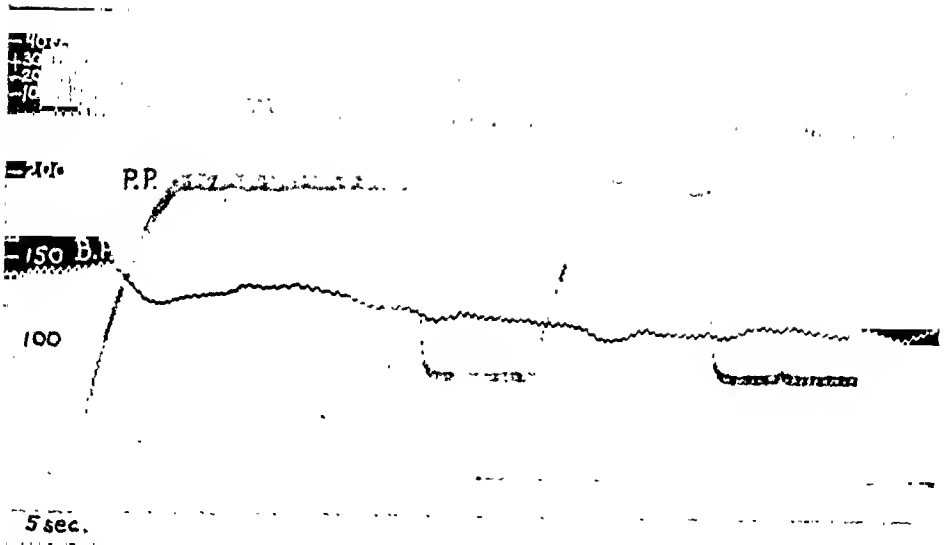


Fig. 10

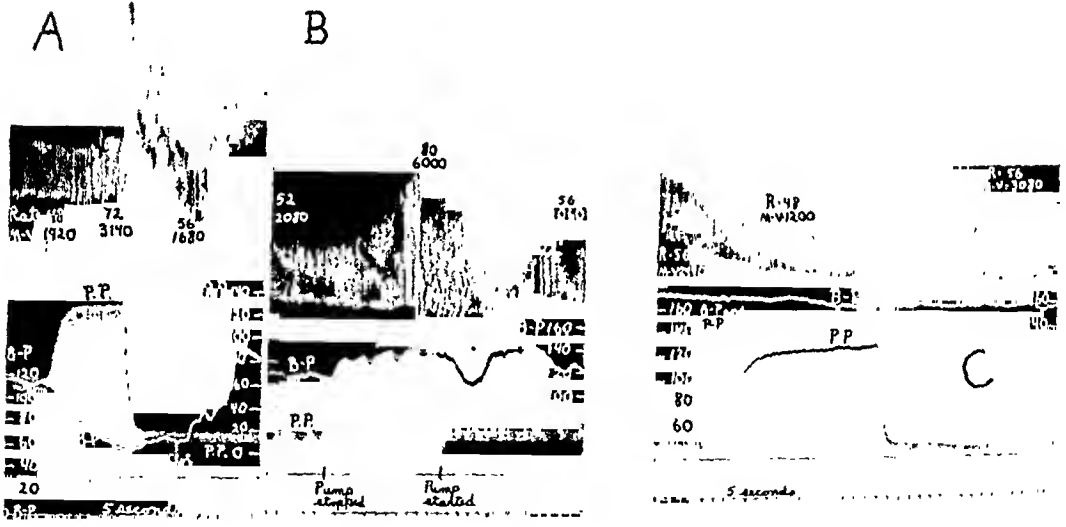


Fig. 11

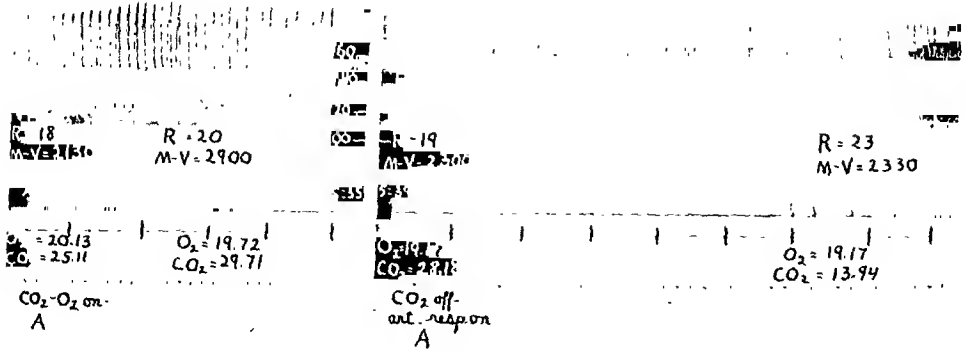


Fig. 15

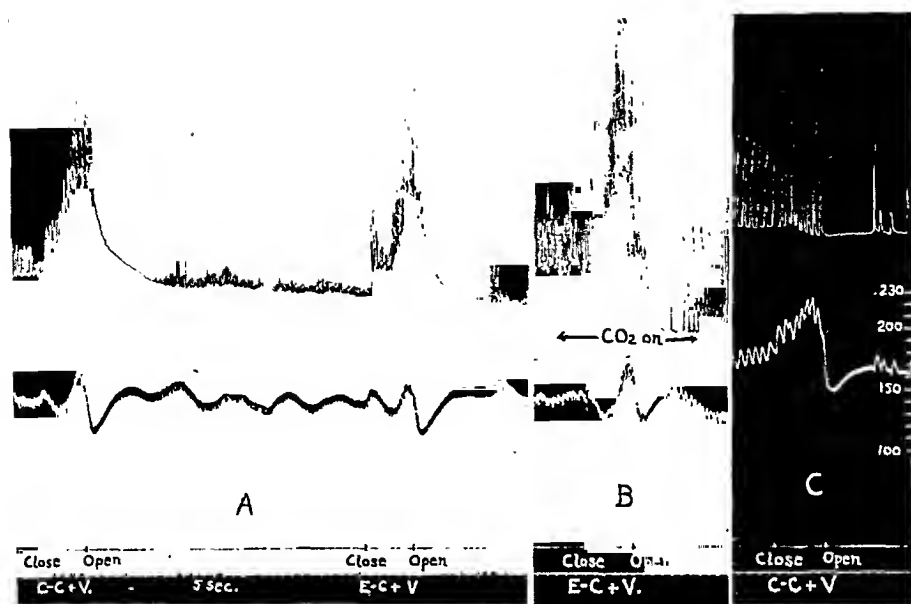


Fig. 2

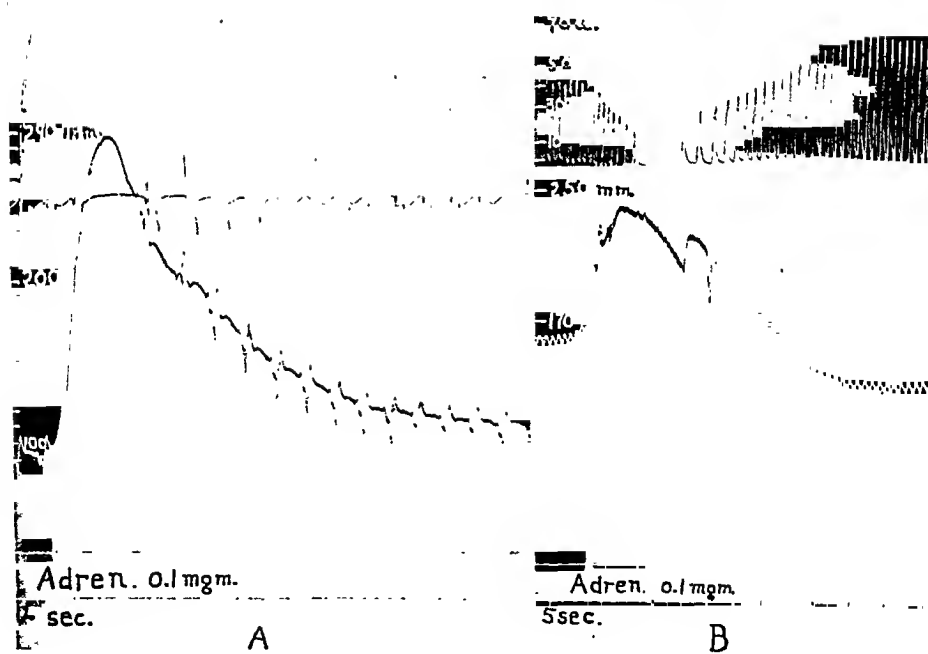


Fig. 5

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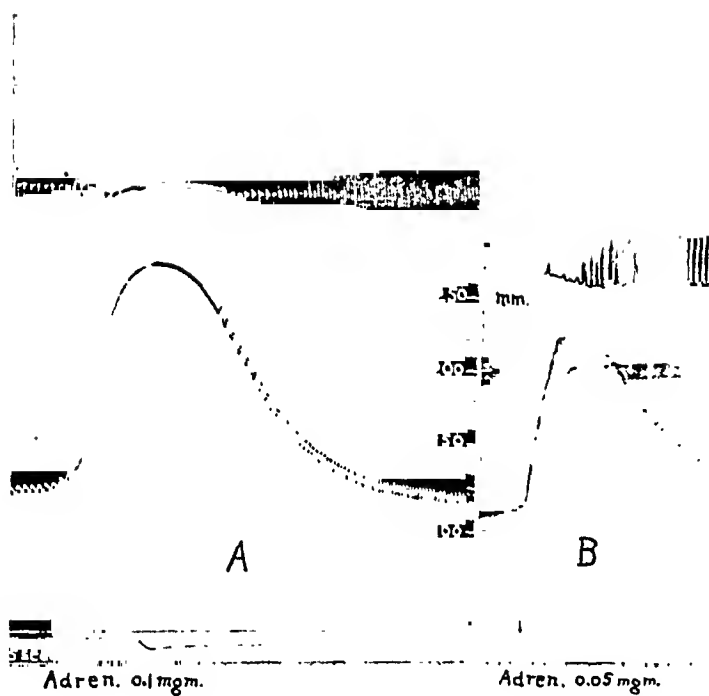


Fig. 6

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THE VITAL CAPACITY OF THE SIAMESE

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Received for publication July 27, 1931

Studies in recent years have indicated that race is a factor affecting vital capacity measurements. As no measurements have heretofore been made upon Siamese, the writer reports the following study made upon a hundred Siamese men. As is well known, age, height, weight, body surface, sex, posture, and state of health are all factors giving variety to vital capacity measurements (Myers, 1925). These factors have been held constant, so far as has been practicable, in making the measurements here reported.

MATERIAL AND MEASUREMENTS. The present measurements were made upon men of ages 21, 22, and 23 years, soldiers in the Siamese army. All were in good health at the time of measurement, as far as was obvious to ordinary observation of their appearance and behaviour. All had been given four hours of drill each day for six months or more before the measurements were made.

The vital capacity was measured in the standing posture. Three measurements were made upon each man and the highest reading was accepted. One hundred men were measured. The men were weighed dressed. All wore similar uniforms. Three of these uniforms were weighed and averaged and the mean was subtracted from the body weight of each man as measured. The difference was accepted as the net body weight. The body surface area was computed from the height and net weight with the aid of the Du Bois chart. The chart was graduated to read to hundredths of a square meter. The vital capacity per square meter of body surface was calculated. The original data are not presented, for lack of space, but those most essential are summarized in tables 1 and 3.

Analysis of data. All statistics are calculated from the original values without grouping. The distribution of the series of vital capacity values was tested for asymmetry and for flatness by calculation of the statistics γ_1 and γ_2 (Fisher, 1925). The first statistic (γ_1) has the value -0.0018

± 0.1652 , representing an asymmetry in which the mode of the series lies at a higher level than the mean; the second (γ_2) has the value -0.6144 ± 0.3304 corresponding to a distribution curve flatter and narrower at the base than the normal curve. If the true distribution of vital capacity measurements were normal, a degree of asymmetry as great as that found here would probably occur due to chance alone in 99 out of 100 series of measurements such as this; and a degree of flatness as great as this sample shows would occur in over 20 out of 100 such series. As there is not sufficient evidence, therefore, to make it probable that the variable departs from the normal distribution, we assume, pending collection of more data,

TABLE 1
Vital capacity frequency

Vital capacity, liters (grouped) (1).....	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0
Number of persons in group (2)...	1	7	9	16	17	12	21	11	4	1	1

TABLE 2
Means and standard deviations

MEASUREMENT	MEAN OF 100 VALUES	PROBABLE ERROR OF THE MEAN	STANDARD DEVIATION OF 100	PROBABLE ERROR OF THE STANDARD
	(1)	(2)	(3)	(4)
Age (years).....	21.5300			
Height (centimeters).....	163.1770	± 0.3936	5.8347	± 0.2783
Weight (kilograms).....	54.7740	± 0.3474	5.1505	± 0.2456
Surface area (sq. M.).....	1.5833	± 0.0063	0.0935	± 0.0044
Vital capacity (liters).....	2.9016	± 0.0273	0.4047	± 0.0194
Vital capacity (lit./sq. M.).....	1.8232	± 0.0143	0.2120	± 0.0102

that vital capacity under the conditions of these measurements is a normally distributed variable.

If vital capacity under the conditions of these measurements is a normally distributed variable, and if the mean and standard deviation of an indefinitely large series ("true" mean and standard deviation) are correctly represented by their estimates from this series of measurements (table 2), about 99 per cent of all values measured upon such material should lie between the limits 1.8575 and 3.9425 liters. None of the 100 values in the present series lies outside these limits. The mean vital capacity of these one hundred soldiers is 2.90 ± 0.0273 liters. On the assumption that such means are normally distributed the probability is estimated to be about 99 per cent that the true mean vital capacity of such material lies between the limits 2.80 and 3.00 liters.

The vital capacity per square meter of body surface was calculated on the assumption that vital capacity is a simple linear function of surface area. Table 3 presents data from which the assumption can be tested.

If the variable is normally distributed and if the true mean and standard deviation of such material are correctly represented by their estimates from this series of measurements (table 2) about 99 per cent of all values of vital capacity per square meter of body surface measured upon such material should lie between the limits 1.2739 and 2.3661 liters per square meter of body surface. With the same probability the true mean should lie between 1.7656 and 1.8743 liters per square meter of body surface.

The coefficient of variation of vital capacity is 13.9 per cent; that of vital capacity per square meter of body surface is 11.6 per cent. There is a decrease in the coefficient of variation of approximately 2 per cent, amount-

TABLE 3
Vital capacity against surface area

Surface area, square meters (grouped) (1).....	1.4	1.5	1.6	1.7	1.8	1.9
Number of persons in group (2).....	3	42	32	17	5	1
Mean vital capacity for the group (3).....	2.5433	2.6957	2.9228	3.2223	3.2840	3.5800

TABLE 4
Correlation coefficients

Weight and vital capacity.....	$\pm 0.4948 \pm 0.0509$
Height and vital capacity.....	$\pm 0.5441 \pm 0.0475$
Body surface area and vital capacity.....	$\pm 0.5864 \pm 0.0443$
Height and vital capacity, weight partialled out.....	$\pm 0.3733 \pm 0.0580$
Weight and vital capacity, height partialled out.....	$\pm 0.2770 \pm 0.0623$

ing to more than a 16 per cent reduction in the variability, when the factor body surface, as it affects vital capacity, is held constant. It would seem therefore that a standard table of vital capacity per square meter of body surface would permit more precise prediction of the result of measurement upon an individual person than would a table simply of vital capacity.

The degree of correlation of vital capacity with the other body measurements made is shown in table 4. The degree of correlation with body surface is the highest and is significantly greater than that with body weight. The fact that the coefficient of correlation of vital capacity with body surface is higher than with other measurements lends support to the practice of expressing vital capacity in terms of liters per square meter of body surface, until a higher correlation is discovered.

DISCUSSION. The vital capacity of these men is lower than that reported for white persons in America. West (1920) reported the normal vital

capacity for the American white male to be 2.5 liters per square meter of body surface. This is nearly 0.7 liter per square meter higher than was found in the 100 Siamese soldiers. Race as apparently a factor affecting vital capacity was also demonstrated for negro children by Wilson and Edwards (1922), Smillie and Augustine (1926), and Roberts and Crabtree (1927). According to all observers the vital capacity is lower in the negro than in the white race. It has also been found to be lower in the Chinese (McCloy, 1927) and Filipinos (Nanagas, 1927).

To what extent these racial differences are hereditary and to what extent due to factors in the habits and environment of the people are questions that remain unanswered.

SUMMARY

1. Vital capacity measurements were made upon 100 Siamese soldiers. The mean vital capacity is 2.90 ± 0.0273 liters, or 1.82 ± 0.0143 liters per square meter of body surface.

2. The distribution of vital capacity measurements such as these is very probably symmetrical, and is assumed to be normal in type.

3. The vital capacity of these men is more highly correlated with body surface area than with height or weight, and seems to have a linear relation to body surface.

The writer expresses his sincere thanks to Dr. E. C. Albritton for suggestions regarding the analysis of data and for other helpful suggestions and criticisms.

BIBLIOGRAPHY

- (1) MYERS, J. A. 1925. The vital capacity of the lungs.
- (2) FISHER, R. A. 1925. Statistical methods for research workers.
- (3) WEST, H. F. 1920. Arch. Int. Med., xxiv, 306.
- (4) WILSON, M. G. AND D. J. EDWARDS. 1922. Journ. Amer. Med. Assoc., lxxviii, 1107.
- (5) SMILLIE, W. G. AND D. L. AUGUSTINE. 1926. Journ. Amer. Med. Assoc., lxxxvii, 2055.
- (6) ROBERTS, F. L. AND J. A. CRABTREE. 1927. Journ. Amer. Med. Assoc., lxxxviii, 1950.
- (7) MCCLOY, C. H. 1927. Arch. Int. Med., xl, 686.
- (8) NANAGAS, J. C. 1927. Bull. Hyg., ii, 752.

EFFECT OF CONFINEMENT ON THE GROWTH OF CHICKEN COMBS AND TESTES¹

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The growth and health of chickens are dependent on inherited characteristics, quality and quantity of feed consumed, environment and freedom from disease. Recently, to control intestinal parasites, the use of wire-floored brooder houses and battery brooders has been introduced. This practice, while successful in the control of certain infestations, has raised serious problems for investigation. Our efforts have been directed toward ascertaining the conditions which would permit the growth of chickens indoors and in coops which limit their activities, that would approximate that of chickens raised under normal farm environment.

At the Kentucky Agricultural Experiment Station an experiment is in progress to determine the effect on the growing chick of confinement under laboratory conditions, as compared with normal farm conditions. With this end in view, 360 one-day-old chicks which came from a common parent stock were divided into three groups of 120 each and placed under the following conditions. Lot 1 was raised in colony brooder houses with free access to grass plots and direct sunlight. Lot 2 was kept for six weeks in a hot-water-heated battery brooder of the usual type, containing a series of five superimposed wire-floored pens, each measuring 36 × 36 inches, 9 inches high and so situated as to receive no direct sunlight. The chicks were then transferred to the colony brooder house used for lot 1. Lot 3 was raised for six months in the battery brooders.

At the age of 10 weeks, the cockerels in lots 1 and 2 were separated from the pullets and placed in a colony brooder house which was similar in every way to that used for the pullets, where they were kept until maturity. The cockerels and pullets in lot 3 were separated and transferred to unheated broiler battery pens, the compartments of which were 36 × 36 inches, 18 inches high.

The same ration was fed the three lots. This had been found experimentally to supply all the necessary food factors for the growing chicks.

¹ The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

It consisted of wheat bran 25, wheat middlings 25, ground yellow corn 25, meat scrap (50 per cent protein) 12, dried buttermilk 10, salt 1 and steamed bone meal 5 parts. Enough of a commercial cod liver oil concentrate was added to make 0.125 per cent of the mash. The mash and a grain mixture consisting of equal parts of cracked yellow corn and wheat were fed ad libitum in hoppers.

The chicks in each lot were weighed and observed individually every two weeks and records were kept of the mortality in each lot and the food consumed.

The chicks in lot 1, raised in the colony brooder house, did not grow quite so rapidly during the first six weeks as the chicks in lots 2 and 3, which were battery-brooder started and battery-brooder raised respectively.

During the twenty-four weeks over which this experiment extended, there was not a marked difference in the weights of the chicks in the three lots, the chicks in lot 3, however, were heaviest until the eighteenth week.



The chicks raised in the colony brooder house and those transferred to the colony brooder house when six weeks old, appeared decidedly more vigorous than those raised in battery brooders until mature.

Near the tenth week it was observed that the combs and wattles of the cockerels in lot 3 that were battery brooder raised during the experiment were distinctly larger than those in the other two lots. In some instances the combs, because of their weight and size, lopped to one side. This condition became more marked as growth progressed, until at the end of the experiment the comb in practically every case covered one eye. In many instances this resulted in the loss of sight in the eye covered by the comb.

The photograph shows a representative twenty-four-weeks-old cockerel selected from lots 1, 2 and 3, respectively, reading from left to right. The cockerels in lot 1, as represented by the cockerel in the photograph, were vigorous, healthy individuals, as were those in lot 2. This showed that the first six weeks of indoor confinement had little effect, if any, on the cockerels in lot 2. In lot 3, as represented by the cockerel in the photograph, the feathering was ruffled and the combs and wattles were approximately twice as large as those of the cockerels in lots 1 and 2.

Rickets did not occur in any of the three lots. Approximately 5 per cent of the confined chickens had slipped tendons or perosis.

TABLE 1

Weights of the 24-weeks-old cockerels and the weights and three greatest dimensions of their combs and testes

BREEDING CAPACITY	NUMBER OF COCKEREL	WEIGHT OF COCKEREL	COMB				TESTES			
			Weight	Length	Height	Thick-ness	Weight	Length	Breadth	Thick-ness
Lot 1—colony raised										
Good	5021	grams 2216	grams 34	cm. 12.3	cm. 6.7	cm. 1.2	grams 8.3	cm. 4.1	cm. 2.0	cm. 1.5
	5313	2030	39	13.2	6.6	1.8	6.0	3.9	1.7	1.4
	5079	2132	42	13.2	6.9	1.9	5.9	3.8	2.1	1.6
	Average	2126	38.3	12.9	6.7	1.6	6.7	3.9	1.9	1.5
Poor	5024	1710	18	11.0	4.9	1.2	3.7	3.1	1.7	1.5
	5289	1951	25	11.2	6.0	1.2	2.8	2.8	1.5	1.5
	5173	1860	22	10.2	5.1	1.5	3.3	2.7	1.6	1.6
	Average	1840	21.7	10.8	5.3	1.3	3.3	2.9	1.6	1.5
Lot 2—Battery started										
Good	5073	1950	17	9.2	5.5	2.0	6.9	3.8	2.0	1.3
	5352	2425	40	12.7	6.3	1.5	4.1	3.8	1.5	1.2
	5138	1895	31	10.9	6.0	1.4	5.1	3.6	1.7	1.4
	Average	2090	29	10.9	5.9	1.6	5.4	3.7	1.7	1.3
Poor	5115	2480	48	13.5	8.0	1.8	5.0	3.5	1.5	1.2
	5176	1803	20	12.3	6.4	1.6	4.1	3.2	2.1	1.5
	5265	2015	32	11.9	7.3	1.5	3.3	2.9	1.3	1.3
	Average	2096	33	12.6	7.2	1.6	4.1	3.2	1.6	1.3
Lot 3—Battery raised										
Good	5160	1580	70	15.2	9.0	2.2	1.2	2.2	1.0	0.7
	5270	1960	104	16.6	10.9	1.4	3.1	3.1	1.8	1.4
	5316	1411	79	14.7	9.0	2.1	3.5	3.0	1.6	1.3
	Average	1650	84	15.5	9.6	1.9	2.6	2.8	1.5	1.1
Poor	5114	1370	62	15.2	9.0	1.5	2.5	2.9	1.5	1.2
	5141	1640	89	16.7	8.7	2.1	4.2	3.0	2.0	1.5
	5375	1256	69	16.0	9.5	1.2	0.5	1.4	0.9	0.5
	Average	1422	73	15.9	9.1	1.6	2.4	2.4	1.5	1.1

At the end of the twenty-fourth week, three cockerels which were determined by external examination to be in good breeding conditions and three

cockerels which by similar examination were determined to be in poor breeding condition were selected from each lot, weighed individually and killed. The combs were cut off, weighed and measurements made of their greatest length, height and thickness. The body was then opened and the testes removed, weighed and measurements made of their greatest length, breadth and thickness. These data are stated in table 1.

Even though the cockerels selected from lot 3 were smaller than those from lots 1 and 2, the average weights and sizes of their combs were more than twice as large. The average weights of the testes of the cockerels from lot 1, raised in colony brooders, were much larger than those of the cockerels from lot 3, raised in battery brooders. The testes of the cockerels from lot 3, besides being smaller, were found to be less moist when cut than those from the other two groups. While it was impossible to estimate accurately the comparative number of spermatozoa in the semen from the three sets of cockerels, it was observed that there was a decidedly smaller number in that obtained from the seminal vesicles of the cockerels in lot 3.

The fertilizing capacity of the cockerels in lot 3 was found to be low as compared with the cockerels in lots 1 and 2 when mated with hens which had previously produced hatchable eggs. This may have been partly due to the smaller number of spermatozoa and to the enlarged combs and partial blindness which unbalanced the cockerels and prevented successful copulation.

The cause of the production of large combs and small testes in cockerels raised in confinement and in the absence of direct sunlight is unknown. It seems possible that the enlarged combs were associated with the exclusion of direct sunlight, the cockerels elaborating combs with larger surfaces to compensate for the decreased quantity of solar radiation received. The development of smaller testes which in two instances were rudimentary may have been due also to the decreased quantity of solar radiation received. It is a well known fact that close confinement is not beneficial for breeding males and that the lack of exercise usually leaves the male somewhat heavier than usual and in poorer breeding ability.

Womack, Koch, Domm and Juhn (1931) have shown that light retards or lowers the comb growth response in Brown Leghorn capons to a given dose of testicular hormone and discuss unpublished data of Dr. L. V. Domm concerning the seasonal variations in the sizes of combs of normal cocks and poulardes which are possibly influenced by light and exercise.

Search of the literature has failed to reveal any previous reference to these phenomena in White Leghorn cockerels. This may be because confinement has been practiced for only a short time or because the males usually are sold as broilers at an early age. Further work along this line has been undertaken to find the effect on the testes of the cockerels when the combs are removed at an early age.

These results show that cockerels raised in confinement in battery brooders, from which direct sunlight was excluded, developed abnormally large combs and smaller testes than those raised in colony brooders, with access to a grass range and receiving direct sunlight.

BIBLIOGRAPHY

WOMACK, E. B., F. C. KOCH, L. V. DOMM AND M. JUHN. 1931. This Journal, xli, 173.

FACTORS INFLUENCING THE PASSAGE OF LIQUIDS FROM THE STOMACH INTO THE INTESTINE¹

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The subject under consideration is not new. In fact, the number of investigators associated with it is so large that in our paper only a brief mention of their accomplishments is possible.

The first and most celebrated of these was William Beaumont (1). The vast number of his careful observations upon Alexis St. Martin are yet regarded as worthy of emulation. Important advances in gastrointestinal physiology were made by Hirsch (2), Marbaix (3), von Mering (4) and Moritz (5) among physicians and Pavlov (6) and Cannon (7) among physiologists. With the name of Marbaix (3) is associated the phrase "test portion." As the stomach is filling, a small amount of the contents passes into the intestine. If it is of the proper reaction and state of digestion the remainder is evacuated promptly. If unfavorable conditions exist, however, the intestine defends itself against invasion either by closure of the pylorus or by anti-peristaltic movements. This investigator was the first to emphasize the importance of the condition of the intestine in gastric evacuation. Especially abundant and valuable material in this field was presented by Pavlov (6) and his school. A study of liquid substances occupied most of their time. Pavlov and Serdiukoff (8) reported that acids are retained in the stomach for a long time. Neutral solutions pass into the intestine very freely, depending upon the evacuation time of that organ. They further found that alkaline solutions do not delay gastric evacuation but on the contrary accelerate it, and pass into the duodenum even more rapidly than neutral liquids such as water.

Pavlov and his pupil Lintvareff (9) established the fact that liquefied fat passes readily into the intestine but is promptly regurgitated into the stomach. Such exchange between the intestine and stomach may occur many times, depending upon the quantity and composition of the fat and the condition of the intestine.

In passing, we must mention the work of one of us (Boldyreff, 10) in 1911

¹ Reported April 28, 1932 at Annual Meeting of American Physiological Society in Philadelphia.

which showed that not only acids and fats but all strong irritants, even alkalies, are retained unusually long in the stomach.

Apperly and Crabtree (11) have recently reported that "the emptying time of the stomach is determined by the pH of the blood."

In such a short résumé, one is obliged to enumerate only the most important contributions to the knowledge of the subject. It is to be regretted that space forbids a further discussion of them.

EXPERIMENTAL PROCEDURES. Of the five dogs used in our observations, four had gastric fistulae. The other had a fistula tube in the duodenum. Dog 27, male, weight 20,700 grams, age 7 years; dog 120, female, weight 12,500 grams, age 7 years, intestinal fistula; dog 145, female, weight 13,000 grams, age 1 year; dog 146, male, weight 13,000 grams, age 1 year, suffered from duodenitis and pancreatitis as confirmed by autopsy; dog 150, male, weight 13,000 grams, age 1 year.

The liquids studied were introduced into the stomach through the fistula tube in 200 cc. quantities. Except in experiments on the influence of temperature, they were warmed to 40°C. before being used. At the end of a twenty minute interval the remaining liquid was removed from the stomach. The results obtained were tabulated for comparison and contrast.

In studying the passage of acids from the stomach, a slightly different technique was used. Two hundred cubic centimeters of acid of known strength were introduced into the stomach. At the end of twenty minutes the residue was measured and reintroduced, a small portion being reserved for titration. This procedure was repeated at intervals of twenty minutes until the stomach was practically empty. Thus the relation between gastric evacuation and neutralization was studied.

The fluoroscope was used in observing the action of the pylorus. An opaque mixture of slightly alkaline (0.5 per cent MgO) starch (2 per cent) and barium sulphate was introduced into the stomach either by gastric tube or through the fistula. Roentgenograms were taken.

The action of irritants can be best divided into two groups, chemical and mechanical. It should be mentioned now that no solutions were used which were too strong to be taken by mouth. All of the liquids were tested on man before being introduced into the dog's stomach. Pepper (0.3 per cent), ginger (1 per cent), currie (2 per cent) and mustard infusions (1 and 3 per cent) and alcohol in solutions of 1 to 15 per cent belong to the class of chemical irritants. In order to avoid any mechanical action these substances were filtered. Nevertheless their inhibitory action was marked. Mustard and pepper prolong the time required to empty the stomach to twice or three times that seen with plain tap water. Little delay is noted when alcohol is administered in dilute concentrations. However, in strengths of 5 to 15 per cent it is strongly inhibitory. The delay is similar

to that observed with mustard and pepper. The action of psyllium seed and bran as a mechanical irritant was studied with considerable interest. Although our number of observations is limited, they appear to confirm the statement that gastric evacuation is hastened by their presence.

Acids have long been known to be slow in leaving the stomach. The inhibitory action appears to be in direct proportion to the strength of the solution; weak lactic acid (less than 0.1 per cent) and HCl (0.05 per cent and less) are evacuated as if they were tap water. Many observations on lactic acid of various higher concentrations (0.1–0.6 per cent) show that they delay evacuation. Such an action is more pronounced with hydro-

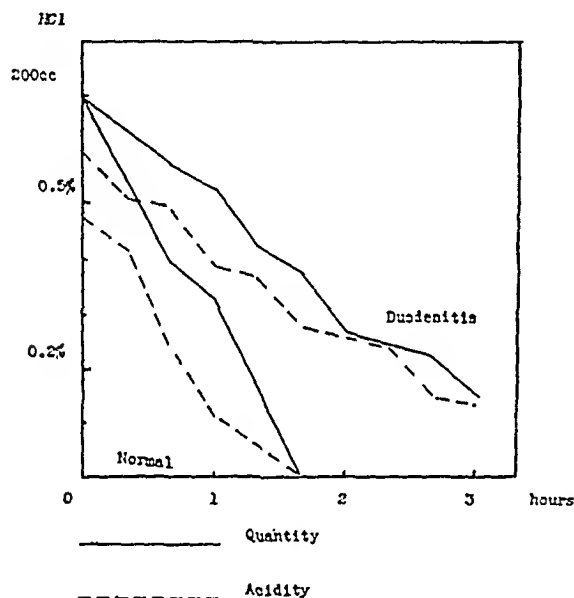


Fig. 1

Fig. 1. Gastric evacuation of HCl

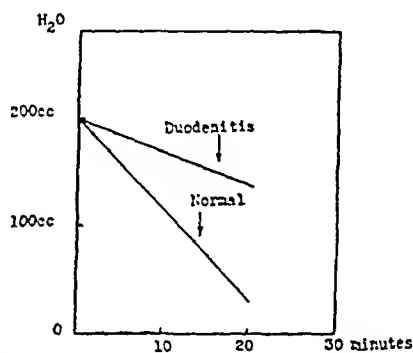


Fig. 2

Fig. 2. Gastric evacuation of water

chloric acid; in a normal dog, it requires one hour to one hour and a half to empty the stomach of 200 cc. gastric juice of 0.5 per cent HCl. A similar amount of water passes into the duodenum in approximately twenty or thirty minutes.

One of the most fruitful portions of our research was that which concerned alkalies. Contrary to the supposition that alkaline solutions leave the stomach more quickly than neutral ones, it was observed that their action was markedly inhibitory. Excepting with very weak solutions (0.5–1 per cent NaHCO_3) this was observed in every case. Substances used were: NaHCO_3 0.1–6.4 per cent, unfiltered calcined magnesium 1–2 per cent, filtered calcined magnesium 2 per cent, filtered lime-water 0.6

TABLE 1

Effect of gastritis on gastric evacuation

Two hundred cubic centimeters of liquids introduced into the stomach for 20 minutes at 40°C.

Tap water*.....	86	50	58	40	60	72	58	78
NaCl, 0.9 per cent*.....	48	83	84					
NaCl, 0.3 per cent*.....	103							
Distilled water*.....	50							
MgSO ₄ , 2 per cent*.....	93							
Na ₂ SO ₄ , 2 per cent*.....	90							
Tap water control.....	30	30	24	19				

* Normal figure for each of these is 20-30 cc.

Remark: All the above figures are obtained on a dog suffering from gastritis, except the last row, tap water control, which refers to a normal animal.

Experiments from October 5 to December 31, 1931.

TABLE 2

Effect of duodenitis on gastric evacuation

Dog N 146. Two hundred cubic centimeters of liquids introduced into the stomach at 40°C.

Tap water, 20 minutes.....	97	92	125	66	134	36
Gastric juice, 0.60 per cent HCl, 20 minutes..	184					
Lactic acid, 0.067 per cent, 20 minutes....	78					
Lactic acid, 0.11 per cent, 10 minutes, {	70					
15 minutes.....	43					
Lactic acid, 0.22 per cent, 10 minutes....	82					
Lactic acid, 0.90 per cent, 20 minutes....	148					
Tap water control, 20 minutes.....	30	30	24	26	30	

Remark: All these figures, except the last line, refer to a diseased animal.

Experiments from November 4 to December 4, 1931.

TABLE 3

Effect of irritants on gastric evacuation

Two hundred cubic centimeters of liquids introduced into the stomach for 20 minutes at 40°C.

1 per cent mustard infusion.....	135					
3 per cent mustard infusion.....	87					
0.33 per cent pepper infusion.....	70					
2 per cent currie infusion.....	81					
5 per cent very old horse radish infusion...	110					
1 per cent alcohol.....	46					
2 per cent alcohol.....	30	30				
4 per cent alcohol.....	22					
5 per cent alcohol.....	74					
10 per cent alcohol.....	68	104				
15 per cent alcohol.....	106					
Tap water control.....	30	30	30	19	21	

Experiments from July 26, 1931, to May 26, 1932.

per cent, filtered MgO 1 per cent and NH_4OH 0.5–1 per cent–1.5 per cent.

The action of some neutral salts was found to be similar in many respects to that of alkalis. NaCl 2 per cent, MgSO_4 2 per cent and Na_2SO_4 2 per

TABLE 4
Evacuation of alkalis

Two hundred cubic centimeters of liquids introduced into the stomach for 20 minutes.

NaHCO_3 , 6.4 per cent, 20°C.....	127					
NaHCO_3 , 3 per cent, 20°C.....	147					
NaHCO_3 , 2 per cent, 20°C.....	100	88				
NaHCO_3 , 1 per cent, 20°C.....	8	34				
NaHCO_3 , 0.5 per cent, 20°C.....	30					
Filtered calcined Mg, 2 per cent, 40°C....	38	54				
Unfiltered calcined Mg, 2 per cent, 40°C...	68					
NH_4OH , 0.5 per cent, 40°C.....	47					
NH_4OH , 1 per cent, 40°C.....	42	64				
Filtered lime water, 0.6 per cent, 40°C....	117					
Filtered MgO, 1 per cent, 40°C.....	51					
Tap water control, 40°C.....	30	24	30	26	30	30

Experiments from October 1, 1931, to January 5, 1932.

TABLE 5
Evacuation of acids

Two hundred cubic centimeters of liquids introduced into the stomach at 40°C.

HCl , 0.60 per cent, 20 minutes.....	196					
Gastric juice, 0.51 per cent HCl , 20 minutes.	139					
HCl , 20°C., 0.2 per cent, 20 minutes.....	125					
HCl , 0.11 per cent, 20 minutes.....	100					
HCl , 0.05 per cent, 20 minutes.....	30	22				
Lactic acid, 0.63 per cent, 20 minutes.....	126					
Lactic acid, 0.22 per cent, 15 minutes.....	78					
Lactic acid, 0.125 per cent, 20 minutes.....	44					
Lactic acid, 0.1 per cent, 20 minutes.....	40					
Lactic acid, 0.07 per cent, 15 minutes.....	40					
Tap water control, 20 minutes.....	30	28	30	30	24	26

Experiments from October 1, 1931, to May 21, 1932.

cent were notably inhibitory. Observations on mineral oil showed it to leave the stomach in approximately the same time as does tap water.

Quinine (0.5 per cent) and peptone (10 and 20 per cent) caused inhibition to gastric evacuation. The action of the former was mild, the latter severe. With 20 per cent peptone only 18 cc. of solution left the stomach in the twenty minute interval. Starch in 2, 3, 4 and 6 per cent solutions appeared to have little action.

TABLE 6
Miscellaneous substances

Two hundred cubic centimeter portions introduced into the stomach for 20 minutes.

NaCl, 4 per cent, 20°C.....	200			
NaCl, 2 per cent, 20°C.....	100			
NaCl, 0.9 per cent, 20°C.....	48*	83*	84*	18
NaCl, 0.3 per cent, 20°C.....	103*	24		
Distilled water, 40°C.....	50*	20	21	20
MgSO ₄ , 2 per cent, 40°C.....	98			
Na ₂ SO ₄ , 2 per cent, 40°C.....	69*	148*	20	
Na ₂ SO ₄ , 4 per cent, 40°C.....	48			
Na ₂ SO ₄ , 6 per cent, 40°C.....	178			
Mineral oil, 45°C.....	22			
Starch, 2 per cent, 40°C.....	38			
Starch, 3 per cent, 40°C.....	44			
Starch, 4 per cent, 40°C.....	40			
Starch, 6 per cent, 40°C.....	40			
Peptone, 10 per cent, 40°C.....	60			
Peptone, 20 per cent, 40°C.....	182			
Egg white.....	38			
Quinine, 0.5 per cent, 40°C.....	37			
Tap water control, 40°C.....	30	30	30	

* These figures are to be explained by the presence of an acute gastritis, as was found later.

Experiments from October 5, 1931, to May, 1932.

TABLE 7
Effect of temperature on evacuation of tap water

Two hundred cubic centimeters of water introduced into the stomach for 20 minutes.

2°C.....	40							
3°C.....	51							
4°C.....	24	45						
6°C.....	36							
8°C.....	72*							
10°C.....	27							
12°C.....	34							
15°C.....	40							
20°C., room temperature.....	30							
40°C., body temperature.....	30	28	30	30	24	26	30	30
45°C.....	70*							
46°C.....	56							
48°C.....	50							

* Large figures are to be explained by the presence of an acute gastritis, as was proven later.

Experiments from October 1, 1931, to March 31, 1932.

It was incidentally found that sometimes apparently normal dogs are suffering from acute gastritis. In such cases the evacuation of different substances was greatly retarded. (See tables 6 and 7.) Even tap and distilled water was retained in the stomach for a considerable period of time.

On one dog, used for experimentation, the results obtained were markedly different from those observed on other subjects. All solutions, even tap water, were retained in the stomach much longer than had been considered normal. Post-mortem examination of this animal revealed the presence of both duodenitis and pancreatitis. (See table 2.)

A similar observation was made on a dog which chewed up his fistular tube and swallowed it. An acute duodenitis ensued and the same phenomena were exhibited as observed in the dog before mentioned. The end results of this latter case were more gratifying as the dog recovered.

In view of the fact that a great deal of importance has been attached to the temperature of liquids entering the stomach, some observations were undertaken to determine the influence of water at varying temperatures on the emptying time of this organ. A great deal of care was exercised in getting a true control group of experiments. A body temperature of 40°C. was chosen as the optimum for the subjects. Tap water² was cooled to 2°, 3°, 4°, 6°, 8°, 10°, 12°, 15° and 20°. At these temperatures there was no case in which evacuation was markedly inhibited. The residues ranged from 24 to 51 cc. as compared with the 30 cc. standard at 40°C. A similar result was noted with temperatures of 45°, 46°, 48° and 50°.

DISCUSSION. The rapidity with which the stomach empties itself of liquids appears to depend upon several factors. The first and most important of these concerns gastric acidity. Acid secretion may result from psychic stimuli or from the presence of specific secretagogues in the viscus. Such excitants as the sight and smell of food can be eliminated but it is more difficult to keep the dogs from misinterpreting certain activities, particularly sounds, in their environment such as preparations for feeding, etc.

There are many substances which excite a definite flow of gastric juice. Among the more familiar ones are: water (tap and distilled), NaCl, alcohol and peptone. Several others which were unknown are: ammonia, MgO (1.5-0.125 per cent), MgSO₄ (2 per cent), and quinine (0.5 per cent). Of these peptone, alcohol and ammonia are the strongest. Doubtless this action sometimes plays a very important rôle in inhibiting gastric evacuation. A solution of peptone had the longest emptying time and was the most active in the production of gastric juice.

² This water was analysed and the following figures were obtained: total solids 0.0372, organic matter 0.0127, SiO₂ 0.0023, FeO 0.0003, Ca 0.0097, Mg 0.0021, Cl₂ 0.0013, SO₄ 0.0020. Parts per 100.

The action of some neutral salts as gastric stimulants is probably the underlying factor in their slow passage into the duodenum. Logically, therefore, the weaker the solution the more nearly it approaches the evacuation time of tap water. As mentioned in the tables, very weak acids and alkalies and some neutral salts do exhibit this property.

Since the discovery of the phenomenon of duodenal regurgitation by one of us (W. N. B.) a great deal of interest has been manifested in regard to its rôle in the regulation of gastric acidity. In our observations upon the evacuation of acids we noticed an interesting phenomenon. After the acid had been in the stomach for twenty minutes there was a marked decrease in acidity but little change in chloride content and volume. In one case there was actually more liquid present than was introduced through the fistular tube. The color was not infrequently yellow and flakes of mucus were also observed. Determinations of chloride content showed that while the acidity decreased very markedly, the fall in chlorides was not parallel. For example: In one hour the acidity fell from 0.51 per cent HCl to 0.20 per cent while the chlorides diminished from 0.53 per cent to 0.46 per cent (experiment of April 12, 1932). Such observations help us to understand more fully the delay in normal gastric emptying and that in the dog suffering from duodenitis and pancreatitis.

CONCLUSIONS

1. Gastritis and duodenitis inhibit gastric evacuation markedly. Even tap water is delayed two or three times the normal limits.
2. No other liquid leaves the stomach more rapidly than water.
3. Strong alkalies inhibit the emptying of the stomach as much as do acids.
4. Weak alkalies leave the stomach with the same rapidity as weak acids.
5. Gastric emptying is prolonged by alcohol, mustard, pepper, etc., some bitter salts and peptone.
6. Temperatures from 2° to 50°C. have very little effect upon the emptying time of the stomach.
7. Duodenal regurgitation is the most important factor in the neutralization of gastric acidity.

BIBLIOGRAPHY

- (1) BEAUMONT. Experiments and observations on the gastric juice and the physiology of digestion. 1833. Plattsburg.
- (2) HIRSCH, A. *Zentralbl. f. klin. Med.*, 1892, 73; *ibid.*, 1893, 377.
- (3) MARBAIX. *La Cellule*, 1898, xiv, 251.
- (4) VON MERING, I. V. *Verhandl. d. XII Kongress f. Innere Medizin.* Wiesbaden, 1893, 471 u. f.
- (5) MORITZ, L. *Zeitschr. f. Biol.*, 1895, xxxii, 56; 1901, B. 52.
- (6) PAVLOV, I. P. *The work of the digestive glands.* London, 1910.

- (7) CANNON, W. B. This Journal, 1907, xii, 387.
- (8) SERDIUKOFF, A. S. Eine der Hauptbedingungen des Uebertritts des Mageninhalts in den Darm. Diss. St. Petersburg, 1899.
- (9) LINTVAREFF, S. I. Ueber die Rolle der Fette beim Uebergang des Mageninhalts in die Darne. Diss. St. Petersburg, 1901.
- (10) BOLDYREFF, W. N. Ergebn. d. Physiol., 1911, xi, 121.
- (11) APPERLY, F. L. AND M. G. CRABTREE. Journ. Physiol., 1931, lxxiii, 331.

THE NUTRITIVE PROPERTIES OF THE "CROP-MILK" OF PIGEONS¹

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In 1786 John Hunter described the material formed in the crops of breeding pigeons for the nourishment of their young, as follows:

The young pigeon, like the young quadruped, till it is capable of digesting the common food of its kind, is fed with a substance secreted for that purpose by the parent animal; not, as in the Mammalia, by the female alone, but also by the male, which, perhaps furnishes this nutriment in a degree still more abundant. . . .

During incubation the coats of the crop in the pigeon are gradually enlarged and thickened, like what happens to the udder of females of the class Mammalia in the term of uterine gestation. On comparing the state of the crop when the bird is not sitting, with its appearance during incubation, the difference is very remarkable. In the first case it is thin and membranous; but by the time the young are about to be hatched, the whole, except what lies on the trachea, becomes thicker, and takes on a glandular appearance, having its internal surface very irregular. It is likewise evidently more vascular than in its former state, that it may convey a quantity of blood sufficient for the secretion of the substance which is to nourish the young brood for some days after they are hatched.

Whatever may be the consistence of this substance when just secreted, it most probably very soon coagulates into a granulated white curd, for in such form I have always found it in the crop; and if an old pigeon is killed just as the young ones are hatching, the crop will be found as above described, and in its cavity pieces of white curd, mixed with some of the common food of the pigeon, such as barley, beans, etc. If we allow either of the parents to feed the brood, the crop of the young pigeons when examined will be discovered to contain the same kind of curdled substance as that of the old ones, which passes from thence into the stomach, where it is to be digested.

The young pigeon is fed for a little time with this substance only, as about the third day some of the common food is found mingled with it: as the pigeon grows older the proportion of common food is increased; so that by the time it is seven, eight, or nine days old, the secretion of the curd ceases in the old ones, and of course no more will be found in the crop of the young. It is a curious fact that the parent pigeon has at first a power to throw up this curd without any mixture of common food, although afterwards both are thrown up according to the proportion required for the young ones.

¹ The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

Claude Bernard (1859) described the phenomenon at greater length and gave the details of an anatomical study of the crop of the pigeon. He found the so-called "crop-milk," or "pigeon-milk," to be composed of desquamated epithelial cells of the mucosa of the crop which clump together to form small masses having the appearance of milk curd. An analysis of the material showed protein and salts 23 per cent, fat 10 per cent, and water 67 per cent; sugar was not found.

A number of studies of the production of crop-milk, and of the histology of the tissues involved, have appeared since Claude Bernard's paper. Among these the work of Phisalix (1890) may be mentioned. Carr and James (1931) and Beams and Meyer (1931) are the most recent workers in this field.

Phisalix, by section of the pneumogastric nerve, had, in one experiment, prevented the proliferation of the mucosa of the crop at the usual time. Recently, however, Riddle and Braucher (1931) have brought about the regular sequence of events, even after section of the nerves, by injection of anterior pituitary extract, and have thereby demonstrated the hormonal control of the phenomenon.

Although considerable study has been devoted to the physiological and histological aspects of the production of crop-milk, little attention appears to have been paid to the somewhat extraordinary nutritive properties of this unique food material. Riddle (1928) has indicated that squabs increase in weight relatively faster than do the young of any other animal for which data are available. This rapidity of growth aroused our interest in crop-milk as a source of energy and of accessory food factors. We have accordingly studied the concentration of vitamins A and B (B + G) in samples supplied to us in daily shipments through the courtesy of Doctors Riddle and Bates of the Station for Experimental Evolution, Carnegie Institution of Washington, at Cold Spring Harbor, Long Island. The specimens were necessarily derived from different birds; they were obtained, in fact, not from the adults, but from the young soon after the crop-milk had been received from the parents. It was found by Doctors Bates that the thin crop wall of the young bird could be slit, the crop-milk removed, and the wound sutured; furthermore that this process could be repeated several times at daily intervals on the same squab without serious injury.

The diet of the adult pigeons consisted of wheat, yellow and kafir maize, and hempseed, together with suitable grits. A calculation of the essential nutrients in this diet indicated the composition: protein (crude) 14.4 per cent, nitrogen-free carbohydrate 62.9 per cent, fat 8.9 per cent, crude fiber 47 per cent, ash 2.2 per cent, water 10.3 per cent; kafir maize and wheat are sources of vitamin B, yellow maize of vitamin A.

The composition of the crop-milk was found, by analysis of pooled specimens, to be: protein ($N \times 6.25$) 18.8 per cent, fat 12.7 per cent, ash

1.6 per cent, water 64.3 per cent. These data are in close agreement with the analysis quoted by Claude Bernard.

At hatching the squab already has some water and a small supply of food in its gut in the form of unabsorbed but modified residual egg yolk. It therefore seemed advisable also to assay the yolk of pigeon eggs for vitamin A, especially as this substance is known to be concentrated in the yolks of the eggs of other species. The eggs of pigeons were therefore also obtained from the laboratory at Cold Spring Harbor; these were heated in water to coagulate the yolks before being shipped.

The ether-soluble portion of the crop-milk was obtained by dehydrating a weighed sample with three successive portions of cold absolute alcohol. The residue was then extracted continuously for 48 hours with hot absolute alcohol and subsequently for about 4 hours with ether. The residual solid was dried in a desiccator, and finally at 90° in an oven in the presence of carbon dioxide, before being weighed. The alcoholic solutions from the cold and hot extractions were combined, concentrated *in vacuo* and freed

TABLE 1

SAMPLE	AMOUNT OF CROP-MILK	SOLID RESIDUE	LIPIDS	
	grams		grams	per cent
1	18.0	14.3	1.696*	9.4
2	30.0	15.4	2.697	9.0
3	46.5	18.6	5.936	12.7

* Lipids from cold alcohol extraction, iodine number 106.2, 106.5. Lipids from hot alcohol extraction, iodine number 114.0, 115.0.

from the last portions of alcohol in a vacuum desiccator at 5 mm. pressure. The lipids so obtained were taken up in absolute ether, the solution was filtered, and the ether evaporated. The lipids were then dried under a stream of carbon dioxide at 90°, weighed, and stored in a refrigerator in small vials under carbon dioxide. The hot and cold alcohol extracts from the first large sample of crop-milk received were worked up separately, and the iodine numbers of the lipids from each were determined by the Hanus method. The results are presented in table 1.

FEEDING EXPERIMENTS. Owing to the limited supply of crop-milk available relatively few animal experiments could be conducted; individual variations have therefore played a larger rôle in this investigation than is ordinarily desirable.

Vitamin A tests of crop-milk and pigeon egg yolk. Twenty male albino rats, weighing 40 to 50 grams at weaning, were fed on a diet, deficient in vitamin A, of the following composition: casein (extracted) 18 per cent, starch 63 per cent, lard 15 per cent, and salt mixture (Osborne and Mendel,

TABLE 2
Results of vitamin A assay of crop-milk and pigeon egg yolk

RAT NUMBER	SUPPLEMENT	AMOUNT OF SUP- PLEMENT	INITIAL		DURATION OF EX- PERIMENT	FINAL	
			Body weight	Ophthalmic condition		Body weight	Ophthalmic condition
		<i>grams</i>	<i>grams</i>		<i>days</i>	<i>grams</i>	
C3910	Crop-milk	0.5	97	Severe	8	77	Severe
C3914	Crop-milk	0.5	96	Severe	12	86	Severe
C3916	Crop-milk	0.5	87	Severe	12	85	Severe
C4033	Crop-milk	1.0	56	Severe	26	103	One eye cured, other nearly so
C4032	Crop-milk	2.0	61	Severe	5	68	Severe
		4.0	68	Severe	10	109	Cured (8th day)
C3900	Ether-soluble frac- tion of erop-milk	0.1	127	Severe	12	126	Severe
C3909	Ether-soluble frac- tion of erop-milk	0.1	112	Severe	8	121	Severe
C3913	Ether-soluble frac- tion of erop-milk	0.1	110	Severe	8	105	Severe
C3918	Ether-soluble frac- tion of erop-milk	0.1	92	Severe	8	85	Severe
C4043	Pigeon egg yolk	0.1	100	Severe	14	115	Cured
C4044	Pigeon egg yolk	0.1	124	Severe	8	132	Severe
		0.2	132	Severe	14	146	Cured
C4045	Pigeon egg yolk	0.1	104	Severe	22	119	Severe
		0.5	119	Severe	13	143	Improved
C4054	Pigeon egg yolk	0.1	109	Severe	8	110	Severe
		0.2	110	Severe	12	120	Severe
		0.5	120	Severe	6	132	Cured
C4060	Whole pigeon egg	3.0	85	Severe	1	96	Cured (6th day)
	Pigeon egg yolk	1.0			1		
	Pigeon egg yolk	0.5			3		
	Whole pigeon egg	0.5			2		
		<i>drops</i>					
C3917	Cod liver oil	1	77	Severe	13	114	Cured (6th day)
C4046	Cod liver oil	1	119	Severe	6	133	Cured (6th day)
C4055	Cod liver oil	1	91	Severe	9	115	Cured (6th day)
C3899	Peanut oil	1	94	Severe	15	68	Severe
C3912	Peanut oil	1	94	Severe	7	72	Severe
C4028	Peanut oil	1	61	Severe	25	52	Severe

At the termination of the experiment 10 drops of cod liver oil were added to the diets of rats C3914, C3916, C3909, C3913, C3918, and C4045. This resulted in a cure of the xerophthalmic condition.

1919) 4 per cent, plus 1 drop daily of maize oil (Mazola) that contained 0.001 mgm. of irradiated ergosterol, and 0.2 gram daily of dried brewery yeast. At the appearance of the usual symptoms of vitamin A deficiency—xerophthalmia and loss in weight—16 to 40 days later, the animals were



Fig. A. 1. Rat C4033, the degree of xerophthalmia obtaining after the administration of 1 gram of crop-milk for 18 days. 2. Rat C4032, a cure of xerophthalmia by 4 grams of crop-milk daily. 3. Rat C4060, the pathological condition of the eyes before the basal diet was supplemented with pigeon egg yolk. 4. Rat C4060, the improvement obtained in 6 days by feeding egg yolk.

divided into groups and the diet was supplemented as is shown in table 2. The crop-milk and pigeon egg were weighed to the nearest centigram and the ether-soluble fraction to the nearest milligram.

The results of these feeding experiments are presented in detail in table 2. They show, with respect to *crop-milk*, that a daily supplementary intake of

0.5 gram was inadequate to insure maintenance of body weight or relief of xerophthalmia; better results were secured with a daily dose of 1 gram; and an intake of 4 grams per day produced a prompt cure.

The *ether-soluble fraction*, when administered in daily quantities of 0.1 gram, or the equivalent of 1 gram of fresh crop-milk, prevented serious declines in weight but had no other apparent beneficial effect; owing to the scarcity of material it was not possible to increase the dosage.

TABLE 3
Results of vitamin B (B + G) assay of crop-milk

RAT NUMBER	SUPPLEMENT	AMOUNT OF SUPPLEMENT	GAIN IN BODY WEIGHT	PERIOD OF TEST	GAIN PER DAY
		<i>grams</i>	<i>grams</i>	<i>days</i>	<i>grams</i>
C4149	Crop-milk	0.5	-6	14	-0.4
C4161	Crop-milk	0.5	+5	14	+0.4
C4162	Crop-milk	0.5	+1	13	+0.1
					Av. +0.1
C4191	Crop-milk	1.0	+30	18	+1.7
C4228	Crop-milk	1.0	+6	21	+0.3
C4229	Crop-milk	1.0	+12	21	+0.6
					Av. +0.9
C4230	Crop-milk	3.0	+17	15	+1.1
C4150	Yeast	0.1	+38	20	+1.9
C4166	Yeast	0.1	+26	18	+1.4
					Av. +1.7
C4163	None		-3	25	-0.1
C4187	None		-9	27	-0.3
C4297	None		-1	27	-0.0
					Av. -0.1

At the termination of the experiment 0.2 gram of dried brewery yeast was added to the diets of rats C4149, C4161, C4162, C4228, C4229, and C4230. This brought about a normal rate of growth.

Pigeon egg yolk produced occasional favorable responses with daily intakes of 0.1 to 0.2 gram. Satisfactory cures were brought about by the ingestion of 0.5 gram a day.

"Positive control" tests with cod liver oil and "negative control" tests with peanut oil gave the respective anticipated responses.

The eye conditions obtaining in some of the animals are shown in figure A: 1 shows rat C4033 after having been fed 1 gram of crop-milk daily for 18 days; 2 shows rat C4032 and illustrates a complete cure of a precious severe ophthalmia by the administration of 4 grams of crop-milk daily for 8 days;

3 and 4 show rat C4060 before and after the administration of pigeon egg yolk, and of whole pigeon egg, for 6 days, and illustrates the marked improvement that resulted.

In general these experiments may be taken to indicate the presence of vitamin A in crop-milk; 4 grams of this material are approximately as effective as 1 drop (30 mgm.) of cod liver oil for the cure of xerophthalmia and the promotion of growth in rats on an otherwise adequate diet. Pigeon egg yolk contains vitamin A in appreciable quantities.

Vitamin B (B + G) tests of crop-milk. Twelve male albino rats, weighing approximately 40 grams at weaning, were given a basal diet consisting of casein 35 per cent, starch 41 per cent, lard 20 per cent, and Osborne-Mendel salt mixture 4 per cent, supplemented by 2 drops of cod liver oil daily to supply vitamins A and D. When the animals failed to gain in body weight the ration was further supplemented as shown in table 3. The results of the feeding tests are taken to indicate that 3 grams of crop-milk supplied approximately the amount of vitamin B (B + G) contained in 0.1 gram of good brewery yeast.

DISCUSSION AND SUMMARY

The nutritive properties of the crop-milk produced by pigeons maintained on a standard mixed grain ration have been investigated; the content of protein, fat, ash, and water was 18.8 per cent, 12.7 per cent, 1.6 per cent, and 64.3 per cent respectively; the material therefore supplies approximately 190 calories per 100 grams. Feeding tests on albino rats have demonstrated the presence of vitamins A and B (B+G) in relatively low concentration; 4 grams of crop-milk were about equal in anti-ophthalmic power to 1 drop of a good grade of cod liver oil, and 3 grams of crop-milk promoted growth at approximately the same rate as 0.1 gram of dried brewery yeast. When fed as a supplement to rats on an otherwise adequate ration crop-milk is therefore a meager source of vitamins A and B (B+G); nevertheless the total quantity of these vitamins presented to the squab, which ingests crop-milk as its sole food, is relatively great. For example, a squab weighing 10 to 13 grams at hatching may ingest 3 to 4 grams of crop-milk the first day, 5 to 10 grams the next, and 10 and 12 grams the following 2 days. The squab has then received the equivalent of 1 drop of cod liver oil and 0.1 gram of dried yeast on the first day, 1.5 to 2 drops of cod liver oil and 0.2 gram of dried yeast on the second day, and 2.5 drops of cod liver oil and 0.4 gram dried yeast on the third and fourth days—a generous allowance of vitamins for so small a bird. Another source of vitamin A for the newly hatched pigeon is the pigeon egg yolk (demonstrated to contain appreciable quantities of vitamin A) which is folded into the gut at hatching.

The liberal supplies of vitamins received by the squab through the daily

ingestion of crop-milk are believed to contribute to the very rapid growth exhibited by these birds.

BIBLIOGRAPHY

- BEAMS, H. W. AND R. K. MEYER. 1931. *Physiol. Zoöl.*, iv, 486.
BERNARD, C. 1859. *Les liquides de l'organisme*, ii, 232, Paris.
CARR, R. H. AND C. M. JAMES. 1931. *This Journal*, xcvii, 227.
HUNTER, J. 1786. *Observations on certain parts of the animal economy*, p. 191; ed. of 1837, p. 149.
OSBORNE, T. B. AND L. B. MENDEL. 1919. *Journ. Biol. Chem.*, xxxvii, 572.
PHISALIX, C. 1890. *Compt. rend. soc. biol.*, xlii, 368.
RIDDLE, O. 1928. *This Journal*, lxxxvi, 248.
RIDDLE, O. AND P. F. BRAUCHER. 1931. *This Journal*, xevii, 617.

ON THE EMPTYING OF THE GALL BLADDER

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While it seems clearly established through the work of Boyden (1925) and others that some evacuation of the gall bladder occurs after a suitable meal, there is a difference of opinion concerning the mechanism responsible for this. On the one hand it is assumed that the important factor is a lowering of pressure with which the full gall bladder has to contend, a pressure regulated through valves in the biliary ducts; or an increase in extra gall bladder pressure due to volume changes in adjacent organs. On the other hand it is believed that the gall bladder through its smooth muscle wall actively contracts and so empties itself. The evidence for the latter belief is based on results from a number of experimental procedures. Thus, it has been shown that isolated surviving gall bladder strips suspended in Ringer's or Locke's solution contract or relax when various drugs or other substances are added to the solution. Again the pressure in an isolated or intact gall bladder may be made to rise appreciably following the administration of smooth muscle stimulants. Finally by direct visual examination, by photographs or by x-rays, it is claimed that actual contractions may be demonstrated.

EXPERIMENTAL METHODS AND RESULTS. In the work reported at this time it was determined what degree of evacuation followed a fat rich meal in cats and whether this could be modified by drugs or other substances assumed to influence the gall bladder contractions. The meal was in most cases readily eaten. In the procedure adopted to measure the fullness of the gall bladder, the cat was killed by chloroform or a blow on the head, the cystic duct immediately clamped and the gall bladder removed. By means of a burette tied into the cystic duct, the amount of fluid added to the gall bladder contents to raise the pressure to a certain fixed level, was measured. A double burette as illustrated in figure 1 was found convenient for this purpose. The pressure used—viz., 100 mm. of water—was that determined by Mann (1919) as the minimum withstood by the common duct sphincter in cats. It is to be noted that there is very little difference in the readings we took at 75, 100, 120 and 140 mm. of pressure; and also that usually no further considerable distention takes place when fluid

is gradually added until 200 mm. of water pressure is reached. The total contents of the viscus were then measured. The amount run in from the burette gave the amount of emptying; and this compared to the total capacity of the gall bladder represented the emptying in per cent.

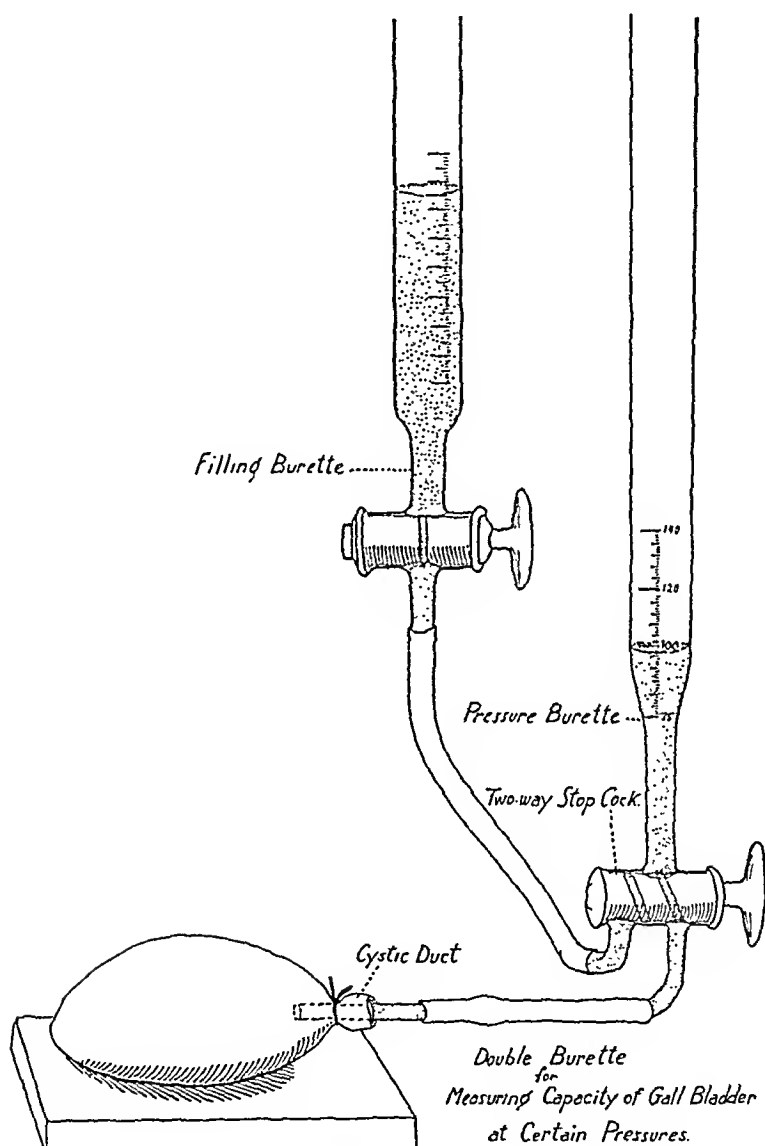


Fig. 1

It was found by this method that the gall bladders of cats not fed for 24 hours or more were filled to about $\frac{2}{3}$ of their capacity. At 4 hours more or less after a meal of 3 egg yolks and 60 cc. of cream, the gall bladders were about $\frac{1}{3}$ full, that is, about $\frac{2}{3}$ empty. When, accompanying the feed-

ing or during the 4 hour period previous to examination of the gall bladder, pilocarpine, atropine, epinephrine, ephedrine or papaverine, were given in single or repeated doses, insuring continued action throughout the period studied and in amounts which were pharmacologically effective, no appreciable change in the degree of emptying could be found either at the end of the 4 hour period or at any time previous. In unfed cats, pilocarpine or histamine in doses sufficient to produce salivation, vomiting, defecation and micturition, did not produce any emptying. The cholecystokin of Ivy also did not cause any measurable emptying.

TABLE 1
Measurement of contents of gall bladders of cats

	GROUP 1 NOT FED FOR 24 HOURS OR MORE		GROUP 2 FAT RICH MEAL, GALL BLAD- DER MEASURED 4 HOURS AFTER MEAL	
	Number exam- ined	Per cent empty	Number exam- ined	Per cent empty
	3	0, 9, 14		
	11	27-38	2	31, 40
	2	43, 45	2	56
	3	60, 63, 63	19	66-90
Atropine.....			4	55, 68, 77, 83
Epinephrine.....			3	68, 71, 71
Ephedrine.....			1	31
			2	75, 81
Papaverine.....			2	40, 56
			3	88, 89, 89
Pilocarpine.....	2	36, 40	2	43, 57
Histamine.....	2	9, 19		
Cholecystokin.....	3	2.5, 27, 30		

In the accompanying table the results are tabulated in two groups. The majority of the gall bladders in unfed cats, group I, are from 27 to 38 per cent empty. It is possible that in the last three of these the starvation period was not long enough. The majority of the gall bladders in the fat-fed cats, group II, are from 66 to 90 per cent empty. Only those cats were used in this series where fat absorption could be demonstrated by the appearance of the white chyle in the lacteals of the duodenum. These figures formed the basis for an analysis of this emptying, when drugs known to activate or inhibit smooth muscle (by acting on the muscle directly or upon its nerve supply) were given in doses shown to be effective in these experiments in eliciting the typical actions in each case. It is apparent that no emptying above the control level could be induced, nor could the emptying resulting from fat be either diminished or increased.

TABLE 2

Dose and manner of administration of drugs

DRUG	DOSE	MANNER GIVEN	TYPICAL ACTIONS ELICITED
Cats			
Atropine.....	0.6 mgm.	Subcutaneous 30 minutes before meal and 90 minutes after	Response of cardiac and gastric vagi to electrical stimuli lost
Epinephrine (1-1000).....	0.5 cc. + 0.25 cc.	Subcutaneous 0.5 cc. 10 minutes before meal; 0.25 cc. 45, 95, 170 and 200 minutes after	Gut relaxed Dose sufficient to produce glycosuria
Ephedrine.....	16 mgm.	30 minutes before meal	Gut relaxed (Kinnaman and Plant, 1931)
Papaverine.....	26 mgm.	Subcutaneous 10 minutes before meal in 2 cases	Gut relaxed
	25 mgm. +	25 mgm. subcutaneous 10 minutes before meal	No narcosis (Macht, 1917)
	12.5 mgm. +	12.5 mgm. 60 minutes after and 12.5 mgm.	
	12.5 mgm.	120 minutes after	
Pilocarpine.....	6-7.5 mgm.	Subcutaneous in controls Subcutaneously 6-11 minutes after meal. Examined after 4 hours	Salivation Emesis Defecation Micturition Gut found tonically contracted with rings of constriction
Histamine.....	3 mgm.	Subcutaneous. Repeated in 30 minutes. Examined after 30 minutes	Salivation Emesis Defecation Micturition Gut contracted in rings
Cholecystokinin..	12 mgm.	Intravenous Femoral vein prepared under local 3 hours previous. This is three times dose given by Ivy	

TABLE 2—*Concluded*

DRUG	DOSE	MANNER GIVEN	TYPICAL ACTIONS ELICITED
Dogs			
Pilocarpine.....	3 mgm. 9-60 mgm.	Intravenously Subcutaneously	Marked tonic contraction rings in gut Salivation Emesis Defecation Pulmonary edema
Physostigmine....	1 mgm.	Subcutaneously	Vigorous peristalsis
Atropine.....	1 mgm.	Subcutaneously	Inhibited actions of pilocarpine and physostigmine
Histamine.....	0.25 mgm. + 0.75 mgm.	0.25 mgm. subcutaneously and 0.75 mgm. after 6 minutes	Marked peristalsis Tremors Defecation
Cholecystokinin..	6-60 mgm.	Intravenously	

Table 2 gives the dosage and the method of administration of the drugs and substances used. The doses in each case, though within physiological limits, were found to be adequate to produce typical effects. The dose of cholecystokinin was three times that used by Ivy.

It would appear, then, that these agents, which under certain conditions can cause contraction or relaxation of gall bladder strips or a rise in pressure in the exposed or the isolated gall bladder, fail to appreciably influence the emptying of the intact gall bladder of unanesthetized cats.

In another part of the work there was a direct visual examination of the gall bladder in unanesthetized dogs. The gall bladder, as well as any other abdominal organ desired, was brought into view by means of what may be called an abdominal endoscope. This instrument illustrated in figure 2 consists of a metal outer sheath of oval cross section accommodating in the upper $\frac{1}{3}$ of its long diameter a combined telescope and light carrier, while the channel formed by the lower $\frac{2}{3}$ allows of the passage of instruments such as electrodes, injecting needles, snares, biopsy punch, endothermy electrodes, etc.

Additional notations to supplement the drawing follow:

The outer sheath is 26 cm. long, oval in cross section with a caliber of 28 F. If no instrumentation is required the caliber of this sheath can be greatly reduced. The distal end is cut at an angle and blunted. The proximal end is fitted with a lock, 2 large stopcocks and a milled disk for holding.

The combined telescope and light carrier employs the McCarthy Foroblique lens system which gives, as the name implies, forward and oblique vision. The telescope is about the size of 14 F (F = French) and affords a brilliant field whose size varies with the distance of the object from the prism, placed just behind the 2.7 volt bulb.

With the endoscope sheath touching the object viewed, the field is 2.5 cm. in radius, so that by rotating the instrument on a perpendicular axis we can examine a field 5 cm. in diameter.

The current for the bulb is supplied by ordinary dry cells with an appropriate rheostat (a cystoscope or bronchoscope battery box does very well) through a flexible cord, which connects with the rotating contact clip.

The telescope is held in the upper third of the long diameter of the tube by passing through a rubber diaphragm in a hollow truncated metal cone, which fits into the lock. The lower portion of the diaphragm is perforated or not according to whether it is desired to introduce instruments through the channel formed by the lower two-thirds of the lumen. The diaphragm is held in place by a metal plate bearing pins

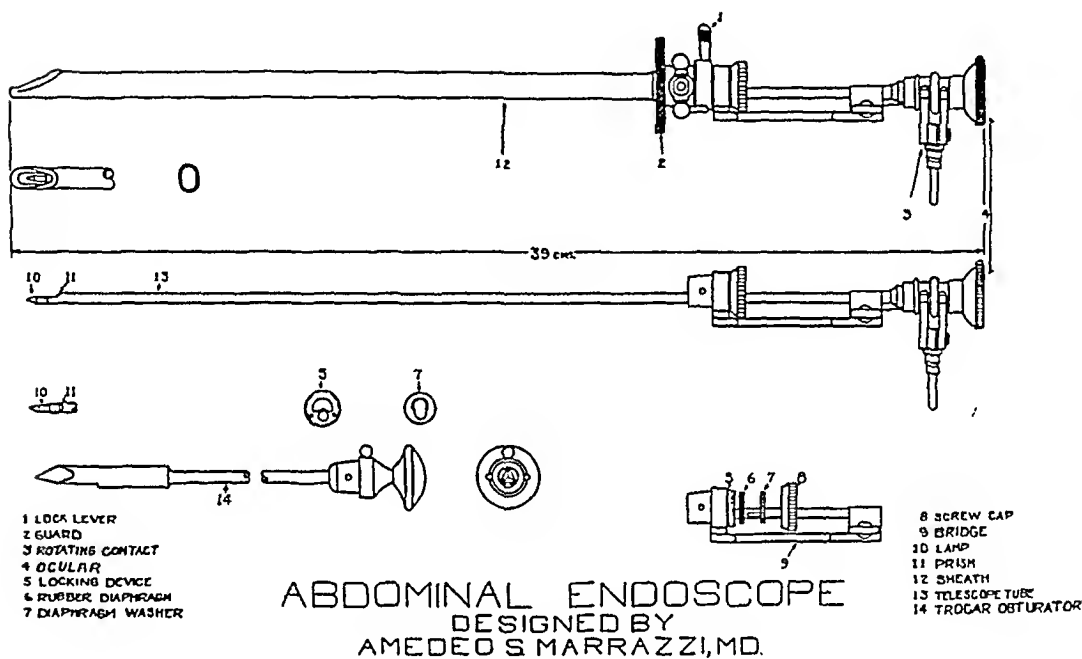


Fig. 2

fitting into the wall of the cone, and perforated with one or two apertures. The plate is held by a screw cap. The whole makes an air-tight joint.

The telescope projects beyond the sheath on its ocular side about 12 cm. to allow of the easy introduction of instruments while the eye is at the ocular. This projecting portion is held rigid by a metal bridge extending back from the cone and ending in an adjustable collar around the telescope just distal to the point of attachment of the rotating contact clip. A pin on the telescope also fits into the collar and prevents any rotation.

The obturator has a three-faced trocar tip with a slot cut out on one side to allow it to slip over the tip of the sheath into which it fits when in use.

Under local novocaine infiltration the endoscope is inserted through the abdominal wall into the peritoneal cavity.

The introduction of the endoscope comprises the following steps:

1. The abdomen of the animal, trained to lie quietly on its back or side

is prepared as for a sterile operation. The chosen site, preferably over some portion of the rectus abdominalis muscle, is infiltrated down to the peritoneum with 1 per cent novocaine. A $1\frac{1}{4}$ cm. skin incision is made. Next, a small amount of air, passed through a sterile cotton filter, is gently blown into the peritoneal cavity through a lumbar puncture needle pushed through the novocainized area. With this air cushion intervening between the anterior abdominal wall and the intestines, the sterilized outer sheath of the instrument, with the trocar-obturator filling the lumen, is carefully pushed through the subcutaneum, muscle and peritoneum, with a steady boring motion.

The telescope is now substituted for the trocar and a minimum of air is gently inflated through one of the stopcocks so as to lift the abdominal wall from the underlying gut and allow a clear view. When properly executed, this is all painless. With some training of the animal and with sterile precautions, observations can be made as frequently and over such periods as are desired, the animal lying quietly on its back and showing no evidence of discomfort. The gall bladder can be examined in any part and with the bright illumination stands out with great clearness.

In a description of the visible changes in the guinea pig's gall bladder following administration of food, Higgins and Mann (1926) report that this organ does not reduce as a unit, but that independent centers of contraction may be seen inducing a variety of patterns in the emptying vesicle. Gradually it assumes a hexagonal and then a pentagonal pattern. They saw no waves of contraction. In the dog, Mann (1932) has been unable to detect any visible contraction. On the other hand, Lueth, Ivy and Kloster (1929), observing directly the gall bladder in two dogs injected with cholecystokinin, saw a circular band of contraction appear at the midportion of the organ with some longitudinal shortening. Later, a similar but smaller band of contraction was seen which appeared and disappeared synchronously with tonus rhythm. It may be pointed out that these two reports describe quite different types of contractions and were made on animals with open abdomens.

In our experiments in which the endoscope was employed, the gall bladder was looked at with great care. Over thirty observations were made on a group of six trained dogs, and a thorough familiarity with the appearance of the organ was obtained. At varying times after a fat rich meal, there was evidence of some diminution in size of the gall bladder, in that there was a falling in of the fundus with some increase in the tortuosity of the vessels, but there were no signs of any actual contraction, either in form of contraction bands or in isolated areas, such as have been described.

Electrical stimulation, by electrodes passed through the tube and applied directly to the gall bladder wall, was without effect, as was also direct

pressure on the organ. Finally in a number of instances, the smooth muscle stimulants, pilocarpine, physostigmine and histamine, were administered in doses sufficient to produce very striking intestinal contraction without gall bladder effect. Cholecystokinine, kindly supplied by Doctor Ivy, also produced no visible contractions.

Discussion. The lack of uniformity in studies on the emptying of the gall bladder appears to us to be largely dependent upon the differing methods employed; and has caused us, after a critical review of the various types of experimental procedures, to employ the two methods here reported.

The difficulty in accepting evidence obtained from isolated surviving preparations of the gall bladder lies not only in the obvious difference between such a gall bladder and one normally in situ, but also in the relatively enormous doses of drugs used. For example, 5 mgm. intravenously in a 15 kgm. dog gives a concentration of 1 to 250,000, while Halpert and Lewis (1930), who use smaller doses than most, have a concentration usually of from 1 to 8000 to 1 to 80,000. Moreover, the drug in such preparations is all available for the gall bladder, whereas only a fraction of that injected into the general circulation reaches it. Furthermore, all except Ischiyama (1925) and later Halpert and Lewis make use of strips rather than the whole viscus, thus failing to elucidate the problem of emptying, while the output recorded by the latter investigators is too small to be regarded by them as significant.

Experiments with the gall bladder in situ in intact animals, on the other hand, suffer greatly from the necessity of subjecting the animal to anesthesia and to the operative trauma of exposure and of cannulating or inserting a balloon into the gall bladder or ducts with concomitant injury to the cystic artery and the nerves running along the ducts. At the same time, the intra-abdominal pressure relations are changed from normal by opening the abdomen.

The results are mainly in terms of pressure variations, which can be recorded in curves showing quite striking rises or falls of short duration. But they actually represent an output of not more than 1.2 cc. and usually about 0.75 cc., according to corresponding experiments of ours. They are further complicated by the action of these drugs upon the liver, and the influence of changes in liver volume upon the gall bladder has been clearly demonstrated by Bainbridge and Dale (1905).

In terms of emptying, then, this type of experiment, excepting in the study of response to fat meals, yields little information and no analysis of the mechanism. It nevertheless appears to be true from the work of Bainbridge and Dale (1905), Taylor and Wilson (1925), Burget (1927) and ourselves (unpublished) that changes of gall bladder tone may be produced by drugs in experiments where the influence of the liver is excluded by

stripping the gall bladder from its fossa down to the cystic duct. The changes, small in extent and transitory, do not so far appear to account for the emptying.

The drawback of reaching conclusions by the methods thus far discussed is illustrated, for example, when contractions of the exposed gall bladder in the intact dog recorded in Ivy's laboratory could not be confirmed in the same laboratory in the isolated surviving organ of the same species, although success is reported by his co-workers, Jung and Greengard (1932), with the isolated guinea-pig gall bladder.

Visualization has, of course, always been desired, and the intravenous dye and x-ray method has been developed to a considerable degree of clinical satisfaction. From the standpoint of physiological investigation, however, it leaves something to be desired. The pictures are at best difficult to interpret. They are, moreover, taken in one plane only, so that changes in other planes, though not otherwise noted, may modify the contour of the shadow appearing on the plate without necessarily denoting a change in volume, thus allowing of misinterpretation.

Lipiodal injection directly into the gall bladder has obviously the disadvantages attendant upon an operative procedure, including trauma of the gall bladder wall. Further, the usual lipiodal is of a higher specific gravity than gall bladder bile, thus complicating the picture by introducing the element of gravity as well as of a higher viscosity.

The definition afforded by fluoroscopy is so vague that, coupled with the objections to x-ray procedure stated above, we can attach little weight to the few reported positive observations: an equivocal one by Pendergrass, Overhalt and Ravdin (1931), who speak of having observed "what we believed to be rhythmic tonic contractions in a human gall bladder" (one case), but then state "It can be said that in these experiments we did not actually see the gall bladder contract," and in the dog, one by Potter and Mann (1926) and one by Ivy and Oldberg (1928).

It was natural despite all difficulties to attempt direct observations of the exposed viscus. This was undertaken by Higgins and Mann (1926) in locally anesthetized animals as already related. They were unable, however, according to Mann (1932), to detect any visible activity of the dog's gall bladder, studied in this fashion.

Ivy and his associates are, therefore, alone in their two reported observations of contractions of the exposed gall bladder in the dog (anesthetized). These are quite different from each other. In one case, when the cystic duct was clamped and the fundus cannulated, contraction bands are described. In another dog whose gall bladder was untouched, much the more physiological experiment, no bands were seen but a gradual reduction in size is reported.

It must be remembered that the gall bladder undergoes a wide excursion

with respiration, with an accompanying distortion of outline resulting from accentuation of the bend normally present at the junction of the neck with the cystic duct, as well as from deformities imposed upon the bladder by taut areas in its peritoneal covering, the occurrence and position of which are very irregular. Again, when the gall bladder is not completely full and is also lax, the contents move within it during its respiratory excursions, causing an undulatory rise and fall of the wall much like a wave of contraction. We mention these deceptive changes, which are artefacts, because others have informed us that their first impression (to be later corrected) at witnessing these changes was one of having seen definite contractions. Another factor to be borne in mind in evaluating such visual observation is the presence of a marked pulsation transmitted from the underlying vessels. Ivy's published photographs and observations are not convincing because of these facts, and because they are made after manually retracting the liver and adjacent viscera. The slightest change in the tension exerted by the retracting hand on the liver, for example, is readily reflected in the gall bladder which is embedded in and attached to it, and would upset any estimation of size or shape as well as comparison with a control photograph.

Our observations, though they differ markedly from those of Ivy and his associates, confirm the work of Friedenwald, Martindale and Kearney (1922) who were unable to observe any active contraction of the gall bladder in 34 dogs under various stages of anesthesia.

The necessity of working with preparations that are less close to the physiological than desired may be largely obviated by the methods of visualization in unanesthetized dogs and the quantitative estimation of gall bladder emptying in previously undisturbed animals that we have reported.

We wish to emphasize that the drugs used in these experiments in attempts to modify the emptying of the gall bladder were given in doses which proved adequate to produce their typical effects to a striking degree. Cholecystokinin was given up to 10 times the effective dose as assayed by Doctor Ivy. The claims made for cholecystokinin have not as yet been confirmed in published reports outside of Ivy's laboratory.

Though muscular activity has been very convincingly shown to play an important rôle in some animals (e.g., the fish), this is not necessarily to be expected in dogs. Thus an examination of the different species used in gall bladder investigation reveals a striking progressive diminution in visible activity from the lower to the higher animals or mammals. Higgins (1928) has reported definite peristalsis of the fish gall bladder; in the guinea pig, Higgins and Mann have seen the distinctly different picture of independent centers of contraction, gradually inducing a hexagonal and then a pentagonal shape in the emptying viscus; while in the dog, they

have been unable to demonstrate contraction visually. In the case of the monkey, Halpert (1927) points out that the gall bladder is normally almost completely embedded in the liver and could not undergo any very striking contractions.

That the dog's gall bladder is not devoid of the possibility of muscular change is indicated by the spontaneous rhythm recorded after cannulization of the gall bladder—freed from the liver—by Bainbridge and Dale (1905), Taylor and Wilson (1925), Burget (1927) and repeated by ourselves, as well as by the slight tonic response elicited by excessive doses of drugs such as pilocarpine. Such type of muscular activity, in its lack of perceptible motion, in its failure to respond to mechanical stimuli (pressure and needle puncture), to electrical stimuli and to otherwise effective doses of drugs influencing smooth muscle, seems very different from that smooth muscle activity, whose function elsewhere in the body is to produce emptying of a hollow viscus. It would seem, therefore, to play a very minor rôle normally in emptying the gall bladder. Its activity appears rather to partake of the nature of a variation of tone which may serve, as suggested by Graham (1926) and Halpert (1929), to effect adjustment of the gall bladder to its varying content. The last is borne out by the appearance of the gall bladder as we have followed it through its cycle of full, tense and smooth; partly empty, lax and wrinkled; same degree of emptying, but not wrinkled and not lax.

SUMMARY AND CONCLUSION

1. The extent of emptying of the gall bladder has been studied by a method of quantitatively estimating the gall bladder bile evacuated in normal, unoperated, fat-fed cats.

2. Gall bladder emptying was studied in unanesthetized trained dogs by a new method of visualization (abdominal endoscopy).

3. The mechanism of emptying has been further studied by means of drugs and substances known to affect smooth muscle directly or through its nerve supply, and by mechanical and electrical stimulation.

4. Direct observation of the viscus during a period when it is emptying failed to show any muscular contraction. An emptying of the gall bladder was not produced or influenced by drugs which have a definite effect on smooth muscle activity, nor by mechanical or electrical stimuli. The conclusion seems warranted, therefore, that muscle contraction in any way comparable to that occurring in other hollow organs, plays little or no part in this emptying.

BIBLIOGRAPHY

- BAINBRIDGE, F. A. AND H. H. DALE. 1905. *Journ. Physiol.*, xxxiii, 138.
BOYDEN, E. A. 1925. *Anat. Rec.*, xxx, 333.

- BURGET, G. E. 1927. *This Journal*, lxxxi, 422.
- FRIEDENWALD, J., J. W. MARTINDALE AND F. X. KEARNY. 1922. *Journ. Metab. Res.*, ii, 349.
- GRAHAM, E. A. 1926. *Amer. Journ. Med. Sci.*, clxxvi, 625.
- HALPERT, B. 1927. *Bull. Johns Hopkins Hosp.*, xl, 390.
1929. *Arch. Surg.*, xix, 1037.
- HALPERT, B. AND J. H. LEWIS. 1930. *This Journal*, xciii, 506.
- HIGGINS, G. M. 1928. *Arch. Surg.*, xvi, 1021.
- HIGGINS, G. M. AND F. C. MANN. 1926. *This Journal*, lxxviii, 339.
- ISCHIYAMA, F. 1925. *Mitt. a.d. med. Fak. d.k. Univ. Kyushu. Fukuoka*, x, 61.
- IVY, A. C. AND E. OLDBERG. 1928. *This Journal*, lxxxvi, 599.
- JUNG, F. T. AND H. GREENGARD. 1932. *Proc. Amer. Physiol. Soc.*, *This Journal*, ci, 61.
- KINNAMAN, J. H. AND O. H. PLANT. 1931. *Journ. Pharm. Exper. Therap.*, xliii, 477.
- LUETH, H. D., A. C. IVY AND G. KLOSTER. 1929. *This Journal*, xci, 329.
- MACHT, D. I. 1917. *Journ. Pharm. Exper. Therap.*, ix, 473.
- MANN, F. C. 1919. *Journ. Lab. Clin. Med.*, v, 107.
1932. Personal communication.
- PENDERGRASS, OVERHOLT AND I. S. RAYDIN. 1931. Cited by I. S. RAYDIN and J. L. MORRISON, *Arch. Surg.*, xxii, 1.
- POTTER, J. C. AND F. C. MANN. 1926. *Amer. Journ. Med. Sci.*, clxxi, 202.
- TAYLOR, N. B. AND M. J. WILSON. 1925. *This Journal*, lxxiv, 172.

THE EFFECT OF CARBON DIOXIDE, HYPERVENTILATION, AND ANOXEMIA ON THE KNEE JERK

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In studies on the lower spinal reflexes reported in an earlier paper (King, Blair and Garrey, 1931), we encountered some evidence that the centers for these reflexes are affected qualitatively by an increase of the carbon dioxide tension in the blood and by anoxemia, as is the respiratory center. We are reporting in this paper the results of studies carried out for the purpose of more fully investigating this point, and also of throwing more light on the mechanism involved in the effects of acid-base changes in the blood on the lower spinal centers, and of evaluating the importance of these changes in accounting for spinal reflex variability within physiological limits.

We have adopted the knee jerk as the criterion, and have studied quantitatively the effects of increasing and decreasing the tensions of carbon dioxide and oxygen in the blood on this reaction. The experiments were carried out to a large extent on barbitalised dogs, but a few decerebrated animals were used. In one group the spinal cord was left intact, but in the other it was sectioned at the level of the last thoracic or first lumbar segment. In about half of the spinal animals the tests were made as soon as a brisk knee jerk could be obtained after the section, but in the others the transection of the cord was done under aseptic technique, and the animals used from four to twelve days later.

Inasmuch as no new principles were involved in the methods of elicitation of the knee jerk and its recording, it is needless to enter into a detailed description of the procedures. Suffice it to say that adequate steps were taken to secure immobilization of the thigh, and also for the control of the stimulus, both as to rate and intensity. The intensity of the stimulus employed was intermediate between minimal and maximal. The few exceptions will be indicated in the text. The blood samples were drawn through a cannula, either from the carotid or from the femoral artery. The respiratory gases were analysed by means of a modified Haldane gas analyser, the blood gases determined by the Van Slyke-Neill manometric method (1924), and the pH by the Cullen (1922) colorimetric method.

THE EFFECTS OF THE ADMINISTRATION OF CARBON DIOXIDE ON THE KNEE JERK. Before quantitative experiments were carried out, it was frequently observed on animals with the spinal cord intact, that the inhalation of a small volume of carbon dioxide resulted in a marked diminution or sometimes in a complete abolition of the knee jerk, while in the spinal animals the result of such a procedure was either no change in the knee jerk (fig. 1), or often an augmentation, and that in order to bring about a complete abolition of the reflex the administration of the gas had to be greatly prolonged. We then inaugurated a series of experiments which we hoped would enable us to arrive at a quantitative expression of this difference.

The carbon dioxide tension of the alveolar air and of the blood was raised by having the animal breathe known percentages of the gas from a Tissot spirometer, and also by rebreathing into a rubber bag of about three

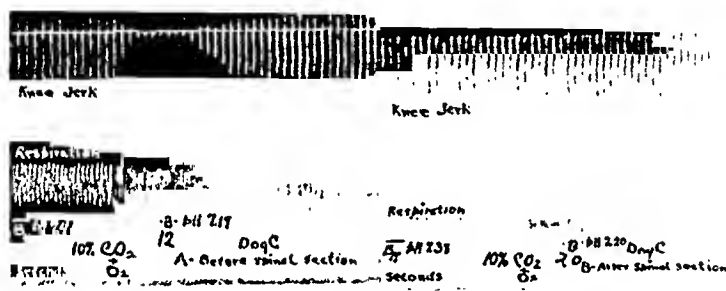


Fig. 1. The effect on the knee jerk of breathing 10 per cent carbon dioxide in oxygen. A, before spinal section, and B, after spinal section. The two records were taken from the same animal (dog C).

liters capacity. On the whole, the experiments in which the animal breathed from a spirometer and exhaled into the room were the most satisfactory. Under these conditions an equilibrium between the blood and alveolar gases is soon established and is maintained at a fairly constant level, while in the case of rebreathing, the level is constantly changing. The animals were allowed to breathe a definite volume of gas, in most of the experiments twelve liters, or the volume necessary to bring about the desired effect on the knee jerk was measured. Blood samples were taken synchronously with the various stages of the reactions obtained. We are fully aware that at any given instant the blood picture with reference to acid-base conditions may not accurately reflect the conditions in the tissues, but it is the most accessible and the best criterion we have, and if the samples are taken in succession and the procedure frequently repeated, the composite picture will at least indicate the trend in the tissues.

The experiments were begun with 2 per cent carbon dioxide in air. In

none of the animals breathing twelve liters of this gas was there any definite change in the knee jerk, although there was a slight increase in ventilation. Three per cent carbon dioxide in air was then tried. In the experiments of Bryan and Garrey (1931) a ten minute period of breathing 3 per cent carbon dioxide in air by a dog in parathyroid tetany resulted in a marked diminution of the clonic movements, but did not abolish the fibrillary twitchings. In our series the effects of breathing twelve liters of 3 per cent carbon dioxide in air were too slight and inconstant to permit any definite conclusions, but there was in every instance a definite increase in the rate and amplitude of respiration. All the gas mixtures containing more than 3 per cent carbon dioxide were enriched with oxygen, in order to guard against an anoxemia entering in as a complicating factor. With the breathing of such a mixture containing 4.5 per cent carbon dioxide in a number of animals with the spinal cord intact there was definite decrease in the knee jerk, but in none of the spinal animals was there any demonstrable effect on this reflex. With further increases in the carbon dioxide tension in the inspired air, in animals with the spinal cord intact, the diminution in response to the patellar tap became progressively more pronounced, so that with a gas containing 8 per cent of carbon dioxide the knee jerk was completely abolished in six out of eleven tests, and with 12.8 per cent it was completely abolished in every instance. In two of these animals a slight but definite augmentation of the knee jerk was recorded just preceding the diminution.

In the experiments with the spinal animals the effects of 2 and 3 per cent carbon dioxide were identical with those obtained on animals with the cord intact, no definite change occurring in the knee jerk, but there was some increase in ventilation. With the breathing of 4.5 per cent carbon dioxide in oxygen, the difference in reactions between the two groups of animals began to be in evidence. In the group with the cord intact, the breathing of 4.5 per cent carbon dioxide resulted, in a significant number of cases, in a depression of the knee jerk, but after transection of the cord there was no change in the reflex. In order to bring about a complete abolition of the knee jerk in the spinal animals, much higher percentages of carbon dioxide were required than in those with the cord intact, the percentages ranging from 9.8, the lowest, to 42, the highest. The type of reaction was also different in more than half of the experiments. Instead of a purely depressant effect on the knee jerk as was the case, with two exceptions, in animals with the cord intact, in more than half of the experiments on the spinal animals a stage of augmentation of the reflex appeared. In some cases there was only augmentation, variable in degree, in others the increased response was followed by a gradual depression. When no augmentation occurred the effect, if any, was one of depression, coming on gradually, and lacking the abruptness characteristic

of the reaction in the series with the cord intact. Figure 2 is illustrative of the augmentatory effect without depression. Another marked difference was noted between the animals of the two series. In those with the cord intact, during the initial stages of depression, and in some at the complete disappearance of the knee jerk, the usual procedures for reenforcement, such as the stimulation of a contralateral nerve, were

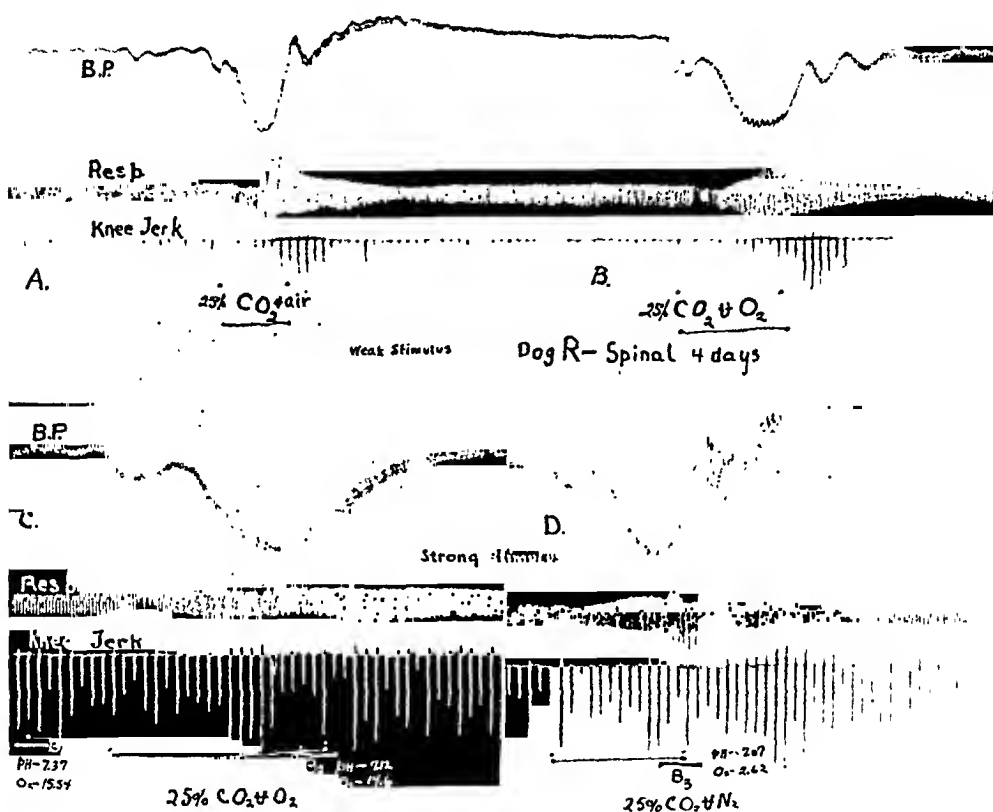


Fig. 2. The augmentatory effect of carbon dioxide on the knee jerk, shown in records A and B, using a stimulus just above the threshold. In records C and D a stimulus near the maximal was employed.

effective, but in the spinal animals they were less effective or failed entirely.

The results of the blood studies revealed very little which could not have been predicted from the analyses of the respiratory gases. They did, however, enable us to separate and discard those experiments in which the carbon dioxide changes were complicated by marked anoxemia, and furthermore threw some light on the cause of the variability between different animals, and also the variability in the same animal as the experiment progressed. No attempt was made to secure uniformity in the

condition of the animals by a course of preliminary care and feeding, consequently the initial control blood samples showed a wide variation in oxygen capacity, pH, and in base. On the basis of our data, no definite pH or carbon dioxide tension level can be correlated with the augmentation, depression, or complete abolition of the knee jerk. The changes indicated in figures 1 and 2 are representative of the average results, and show the differences between the spinal animals and those with the cord intact. Referring to figure 1, dog C, it is seen that before spinal section a change in pH from 7.41 to 7.19 was associated with an almost complete disappearance of the knee jerk, and that after spinal section a drop in pH of almost the same magnitude, from 7.38 to 7.20, was without effect on the reflex. In the records from dog R (fig. 2), the cord having been sectioned four days previously, a change from a pH of 7.37 to 7.12 (section C) shows no alteration in the knee jerk, while the same procedure (section B), with a stimulus just slightly above the threshold, shows a definite augmentation. On the whole, in animals with the cord intact, a drop of from 0.15 pH to 0.20 pH was necessary to bring about complete abolition of the knee jerk, while in the spinal animals a change approximately twice as great was necessary.

The results of these experiments indicate that the initial depression of the knee jerk in animals with the spinal cord intact, on raising the carbon dioxide tension of the alveolar air and of the blood, is not primarily due to a direct depression of the centers in the cord, but is dependent upon an effect on centers situated higher up in the neuraxis. They also indicate that the initial effect of raising the carbon dioxide tension in the blood is to increase the excitability of the lower spinal centers, but not to a degree comparable with the effect on the respiratory center. The depressant effect on the knee jerk, when the carbon dioxide tension is raised to a very high level, a result common to both groups of animals is, in all likelihood, due to direct depression of the lower spinal centers.

The assumption that the initial depression of the knee jerk in animals with the spinal cord intact, resulting from the administration of carbon dioxide, is not a direct one, raises the question as to the mechanism involved. With the data at hand the answer cannot be final. Presumably the effect is due to inhibitory influences from centers high up in the neuraxis, which may be excited either directly or reflexly. In order to throw light on these possibilities, studies were made on decerebrated animals, and on animals after double vagotomy.

In carrying out the decerebration the skull was trephined, then with a knife the brain was transected. On autopsy it was found that the line of division was fairly uniform, passing through the brain stem between the superior and inferior colliculi. The four animals thus prepared manifested considerable rigidity, or were easily thrown into the rigid state. On ad-

ministering air rich in carbon dioxide to these animals, the knee jerk behaved as it did in the spinal animals, not showing any sharp early diminution, but an augmentation in some cases, and disappearing only with the higher concentrations or after prolonged administration. These results indicate that carbon dioxide has a primary excitatory effect on centers, perhaps cortical, which in turn exert an inhibitory influence on the lower spinal centers. On decerebration the lower centers are released from these effects.

The possibility of another inhibitory factor must be taken into consideration, although it may play only a minor rôle. We have shown in earlier work (1931), and it also has been reported by others (Johnson and Luckhardt, 1927), that stimulation of the central stumps of the vagi in dogs exerts a marked inhibitory effect on the knee jerk. We have also demonstrated that under certain conditions there is a definite augmentation of the knee jerk synchronous with inspiration. The response to an increase in the carbon dioxide tension in the blood on the part of the respiratory mechanism is an hyperpnea. Thus, following the administration of carbon dioxide, two factors are released as the result of the increase in respiratory activity, one, an irradiation of impulses from the respiratory center which has an augmentatory effect on the knee jerk, and the other, an increase in afferent impulses from the lungs by way of the vagi, which have an inhibitory effect. It is not unreasonable to assume that under certain conditions the inhibitory effects brought about through the vagal afferents may balance or even outweigh the augmentatory effects from the respiratory center itself. This hypothesis receives support from our observations that the initial depressant effect of raising the carbon dioxide tension in the blood has, on the whole, been more marked in animals with the vagi intact.

The possibility of relations between variations in the knee jerk and circulatory changes has not been overlooked. Records of carotid blood pressure were taken in the majority of our experiments. The mean carotid blood pressure in our animals ranged from about 100 mm. to 140 mm. of mercury, and it was only when the pressure fell to very low levels, 30 mm. to 50 mm., and was maintained at that level for some minutes, or kept gradually falling, that any definite change in the knee jerk appeared. Under these conditions the reflex became increasingly more difficult to elicit. Our results indicate clearly that the variations in the knee jerk which we have described, resulting from the administration of carbon dioxide, cannot be correlated with variations in the mean arterial blood pressure. This is in keeping with the findings of Porter (1912) who studied the variations in the irritability of the spinal centers under asphyxial conditions.

THE EFFECTS OF HYPERVENTILATION ON THE KNEE JERK. Along with

the studies on the effect upon the knee jerk of an increase of the carbon dioxide tension in the blood, we have also studied the effects of its decrease. In order to carry this out we resorted to the expedient of hyperventilation. On the basis of a rather extensive literature it is evident that associated with an alkalosis, some parts, at least, of the central nervous system manifest a considerable degree of hyperactivity. Grant and Goldman (1920) were able to induce severe tetany in some human subjects by hyperventilation, Rosett (1924) reported similar findings, and Lennox and Cobb (1928) state that in epileptics a seizure can often be precipitated by the induction of an alkalosis. In our own laboratory, Bryan and Garrey (1931) were able to bring on violent tetany in parathyroidectomized dogs by this procedure. Our main interest has been to determine the effects of hyperventilation upon the knee jerk, using a stimulus of constant intensity.

In these experiments, just as in those described in the preceding paragraphs, two groups of animals were used, in one the spinal cord was left intact, and in the other it was sectioned. In order to carry out the hyperventilation, the animals, after barbitalisation, were placed into an airtight chamber enclosing the head, thorax, and the upper half of the abdomen. The tracheal cannula was connected to a short tube sealed into the wall and leading to the outside of the box. The hind quarters were drawn through an opening in a heavy rubber diaphragm, thus making these parts easily accessible for the study of the knee jerk. The interior of the chamber was then connected with a suction pump and a pressure tank through a system of dual valves operated by a motor driven speed reducer, timed, so that a fixed degree of negative pressure could be alternated with an equal degree of positive pressure. By the proper timing and the adjustment of the fluctuations of pressure within the chamber, overventilation could be carried out without a fall in blood pressure of sufficient magnitude to account for changes in the knee jerk.

The results of our experiments on animals with the cord intact were quite variable; in some instances the hyperventilation resulted in no change in the knee jerk, at other times a definite augmentation was obtained, and then again, a definite diminution, at times coming on very sharply with the beginning of the hyperventilation, and sometimes developing gradually (fig. 3). Numerous blood samples were drawn at various stages of the experiments, and determinations of the pH, carbon dioxide content and tension, and oxygen saturation made. The blood pictures of the acid-base conditions and the oxygen saturation were quite uniform, and showed no correlation with the knee jerk. The results with the spinal dogs were more uniform, and while there was no quantitative parallelism between the degree of alkalosis and the knee jerk, yet in the majority of instances the reflex was augmented under these conditions.

A number of parallel observations were made which may contribute to

the explanation of the variability in the knee jerk during and immediately following hyperventilation. In the first place, in a number of instances, an abrupt diminution in the knee jerk set in with the beginning of the artificial ventilation, and then just as abruptly returned to the original or higher level when the ventilation was stopped. In the second place, while we were able to produce tremors and tetany in less than half of our animals, in no instance did this condition appear posterior to the level of the spinal

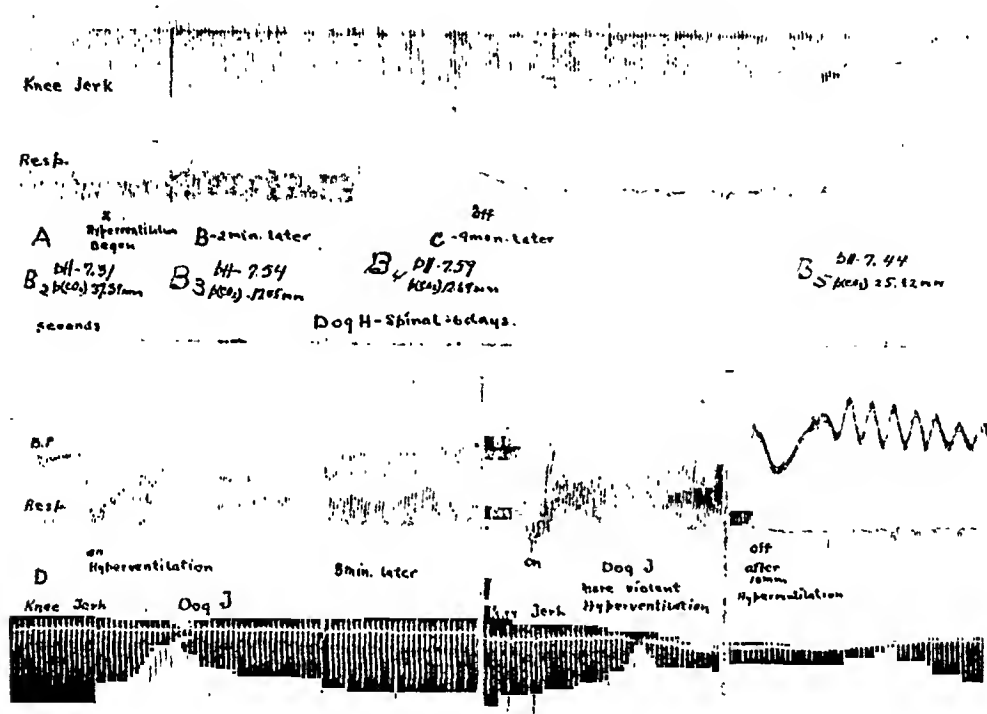


Fig. 3. The effect of hyperventilation on the knee jerk. Sections A, B, and C, taken from a spinal animal, show an augmentation. In D, from an animal with the cord intact, and with mild hyperventilation, no definite effect is shown. In E, taken from the same animal as D, but with more violent ventilation, a diminution is shown. In this case the extensor tone was increased, as shown by the drop in the base line. At B2, B3, B4 and B5 blood samples were drawn.

section. In the third place, the two decerebrate dogs (without spinal section) studied were easily thrown into tetany and rigidity by hyperventilation, and uniformly responded with an augmented knee jerk. These experiments, however, were usually of short duration, because with the onset of rigidity and the maintenance of the rigid state, studies on the knee jerk were rendered practically impossible. In the fourth place, after sectioning both vagi the effects of hyperventilation on the knee jerk were more uniformly in the direction of augmentation, but not always so.

These results and observations indicate that the primary effect of hyperventilation on the spinal centers consists in an increased excitability, but of a mild degree. In this case, and throughout this paper, we have used the terms excitability and irritability in a general sense, in terms of response to a constant stimulus, rather than in terms of threshold, no attempts having been made to determine the least stimulus in any instance. In the intact animal, however, the situation is a complicated one. A state of increased extensor tone and rigidity may set in which will greatly limit the excursions of the leg and present the picture of a diminished response (fig. 3, section E), or the direct effects on the cord may be either reënforced or inhibited by impulses from centers higher up in the neuraxis, the end result being an algebraic summation of these effects, and hence will depend upon the dominant factor. We feel justified in the conclusion that hyperventilation directly leads to an augmented excitability of certain cortical centers, the impulses from which have an inhibitory effect upon the lower spinal centers. An effect of this kind would aid in accounting, in part at least, for the depression of the knee jerk in some animals, and for the fact that it is not possible to demonstrate tetany in all waking human subjects by hyperventilation, and also for the relative ease with which the decerebrate animal is thrown into rigidity and tetany.

Another group of factors, some augmentatory, and some inhibitory, and reflex in character, must be given consideration. Without having direct evidence, one assumes that the increased pulmonary excursions and changes in the great veins in the thorax during hyperventilation give rise to an increase in the number of afferent impulses by way of the vagi. Stimulation of the central stumps of the vagi results in a diminution of the knee jerk, consequently the diminution in the knee jerk sometimes encountered during hyperventilation may, in part at least, be the result of vagal afferent impulses. Whether the result is dependent upon the pulmonary vagal afferents alone, or also upon the cardiac and gastric paths is not known. Impulses from the stomach may have an augmentatory effect upon the knee jerk, for Carlson (1916) found the knee jerk augmented during hunger contractions. It is also possible that the skeletal musculature involved in the thoracic and abdominal movements incident to respiration, even if artificially stretched, may give rise to impulses which enter into the picture and play some rôle, most likely augmentatory in character.

Thus, in the intact animal, the final effect of hyperventilation on the knee jerk is the resultant of the interplay of a number of opposing factors. By decerebration, the cortical inhibitory factors are eliminated, while by spinal section both the cortical influences and those arising lower down are erased. With the spinal centers thus isolated, the effect of hyperventilation upon them is that of a mild augmentation of excitability.

While an augmentation of the knee jerk in the spinal animal cannot be

demonstrated in 100 per cent of the cases, on increasing the carbon dioxide tension in the blood and on hyperventilation, yet it occurs frequently enough to be of significance and, it seems to us, to warrant the conclusions we have drawn. We are thus confronted with a dilemma in which, either by increasing or by decreasing a given factor, the same end result is attained. Walshe (1923) has contended that the hyperexcitability of the central nervous system resulting from hyperventilation is due to tissue anoxia. If this were true, then the dilemma would vanish. His assumption is, that owing to the removal of carbon dioxide the oxygen is not released from the hemoglobin, consequently the tissues actually suffer from oxygen deficiency, and become more acid. This would point to the H ion as the determining factor, being increased, on the one hand, by piling up of carbon dioxide, and on the other hand, as the result of the failure to oxidise lactic acid. Laying aside the improbability, on the basis of dissociation, of this hypothesis, we have accumulated evidence that hyperventilation is not associated with tissue anoxia. Many of our experiments were carried out with the animal breathing into a metabolimeter, and the oxygen consumption determined before, during, and after hyperventilation. If a tissue anoxia supervenes upon hyperventilation, it should be reflected on the oxygen consumption curve. In no instance have we recorded a decrease in oxygen consumption under these conditions, but on the contrary, in a few experiments, an actual increase. Tissue anoxia is therefore ruled out as an explanation, and in the absence of any other conclusive data, we must leave the question an open one.

THE EFFECT OF ANOXIA ON THE KNEE JERK. Sherrington (1910) observed that a slight degree of asphyxia facilitates the elicitation of the scratch reflex in the decapitate and in the spinal animal. Porter (1912) studied the effect of anoxia on the threshold for the flexion reflex on stimulation of the tibial nerve in the spinal animal, and found the effect predominantly depressant. Winkler (1929) reported an augmentatory effect on the reflex contraction of the anterior tibial muscle as the result of anoxia, but he used no spinal animals, and since he introduced a soda-lime cartridge into the respiratory circuit, it is quite likely that the anoxia was complicated by some degree of alkalosis.

We have made a study of the effects of various degrees of anoxia on the knee jerk of animals with the spinal cord intact and also with the cord transected; we have also controlled, within limits, the carbon dioxide tension in the blood during the period of oxygen lack. Instead of using a closed circuit containing a soda-lime cartridge, we prepared mixtures of nitrogen, oxygen, and carbon dioxide in a Tissot spirometer, from which the animal inhaled, and then exhaled into the room. By adding between 2 and 3 vols. per cent of carbon dioxide to the inspired gas, the blood changes in pH and in carbon dioxide tension were less than those found necessary to produce any demonstrable effect upon the knee jerk.

The effects of anoxia on the knee jerk in both groups of animals were predominantly depressant, but quite variable, and may be grouped under three heads,—initial, intermediate, and terminal. We were not able to demonstrate any definite effect on the knee jerk with gas mixtures containing 12 per cent or more of oxygen, but with 10 per cent there was some change in a few animals, and with 8 per cent and less there was always a definite effect.

In about 30 per cent of the experiments on animals with the spinal cord intact, and in about 45 per cent after sectioning the cord, the initial effect of anoxia was an augmentation of the knee jerk, mild and of short duration, but definite. These figures are slightly higher than those given by Porter (1912) who studied the effect of anoxia on the threshold for a flexion reflex, and found it lowered in eleven out of forty-seven cases. Since the duration of the period of augmentation is brief, and his tests were made at intervals

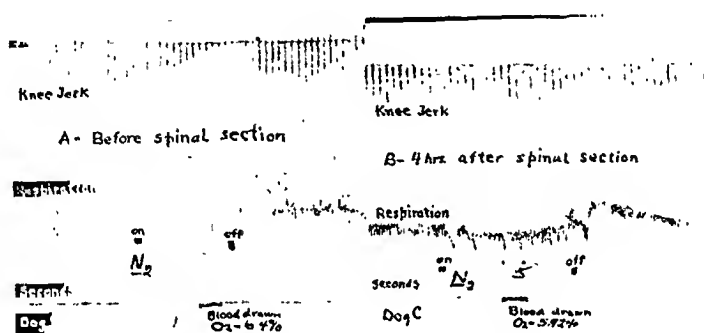


Fig. 4. The effect of moderate anoxia on the knee jerk. A, before spinal section, and B, after spinal section. Both records were taken from the same animal (dog C), and the anoxia was of about the same degree in both cases.

of several minutes, it is not unlikely that had he made continuous records or more frequent tests, he would have found a higher incidence of augmented excitability.

The intermediate stage was invariably one of depression of the knee jerk, ranging from a slight diminution in the reflex to its complete abolition. The degree of depression in any given experiment ran closely parallel with the severity of the anoxia. Blood samples were taken at various points, corresponding in time as closely as possible to various stages of the change in the knee jerk. The results of the analyses of these blood samples revealed the fact that an anoxia of greater severity is required to abolish the knee jerk in the spinal animal than in one with the cord intact (fig. 4), the average, on the basis of oxygen saturation, being 34 per cent for the intact animals, and 15 per cent for those with the cord sectioned. These results indicate that, just as in the case of raising the carbon dioxide tension

in the blood of animals with the spinal cord intact, impulses from centers situated higher up in the neuraxis enter into the picture, and play an important rôle in determining the effect of anoxia upon the knee jerk.

When the anoxia was pushed to the extreme, we frequently encountered the phenomenon which we have designated as the terminal reaction. At this stage the knee jerk was no longer elicitable with the stimulus previously effective, the blood pressure was rapidly rising, the respiratory efforts were slow and of the gasping type and soon ceased entirely. The arterial pressure kept rising for a few seconds after the cessation of respiration, then broke sharply and began to fall rapidly. When this stage was reached, a few of the animals, if disconnected from the Tissot and left to themselves, recovered, but in the majority the situation rapidly became more critical, and demanded the institution of measures for resuscitation, at times successful, and at other times, not. Almost synchronous with the beginning of the fall in blood pressure the knee jerk again became elicitable, rapidly increased in height for four or five responses, then declined in the same manner. At this time the animal usually made a few gasping efforts, and if it rallied from this point and recovered, the knee jerk soon again became elicitable and gradually recovered its normal intensity, but if the animal failed to rally, no more reflex responses were obtained. In so far as the knee jerk is concerned the phenomenon which we have just described is not dependent upon the integrity of the connections between the lower spinal centers and those higher up, for it occurred just as frequently in spinal animals as in those with the cord intact.

On the basis of our results, we feel that the augmentation of the knee jerk associated with the early stages of anoxia occurs frequently enough to be of some significance, and are justified in the conclusion that there is some increase in the excitability of the lower spinal centers, but not to a degree comparable with the effect of anoxia upon the respiratory center. If the anoxia is intense or prolonged, the final effect is always one of depression which may reach the state of complete reflex inexcitability. The recovery from this state is dependent upon the duration and the intensity of the anoxia. In the extreme cases which we have described, in which a brief paroxysm of knee jerks appears following the cessation of breathing and synchronous with the break in blood pressure, the interpretation and analysis are somewhat difficult. Blood samples taken at this stage have invariably shown a very low oxygen content, 0.92 vol. per cent being the lowest from which recovery took place, and a carbon dioxide tension higher than that found as the norm. We have also been much more successful in resuscitating these animals by the use of carbon dioxide and oxygen, than by the use of oxygen alone. Our hypothesis is, that the respiratory and spinal centers, as a result of the anoxia, become so depressed that they

no longer respond to the stimuli at hand. With the cessation of respiration the anoxia becomes more intense, but at the same time the carbon dioxide tension in the blood gradually mounts, and finally reaches a level which may be sufficient to set the respiratory center into activity and to raise the excitability of the spinal centers to the point where the patellar taps become effective stimuli. This is the critical point, and if the tissue acidity has not been too great the animal may gradually rally and recover. On the other hand, the tissues may have become so acid that the slightly added increase in the acidity of the blood due to the oxygenation of the hemoglobin, and of the tissues themselves as the result of their activity, renders recovery impossible.

SUMMARY AND CONCLUSIONS

1. In the dog with the spinal cord intact, the initial depression of the knee jerk which results from an increase of the carbon dioxide tension of the blood, is not due to a direct depression of the spinal reflex centers, but to an inhibitory effect from higher centers, due in part to their direct excitation by the carbon dioxide, and in part to reflex excitation.

2. The effect of hyperventilation on the knee jerk is that of a mild augmentation, but in animals with the cord intact, inhibitory influences from higher centers may dominate and lead to an actual diminution. The actual excursion of the leg may also be diminished because of the development of a state of rigidity and increased extensor tone. There is no evidence that the effects of hyperventilation are due to tissue anoxia.

3. In the early stages of anoxia there is evidence of a short period of increased excitability of the lower spinal centers. The effects of a severe and prolonged anoxia are always depressant.

4. The lower spinal centers are qualitatively affected by an increase in the carbon dioxide tension in the blood and by anoxia as is the respiratory center, but quantitatively are much less responsive.

5. The effects of acidosis, alkalosis, and anoxia, within physiological limits, play but a small rôle in accounting for the variability in spinal reflex responses, and are largely overshadowed by other inhibitory and augmentatory factors.

BIBLIOGRAPHY

- BRYAN AND GARREY. 1931. *This Journal*, xcviii, 194.
CARLSON. 1916. *The control of hunger in health and disease*, 86.
CULLEN. 1922. *Journ. Biol. Chem.*, lvii, 501.
GRANT AND GOLDMAN. 1920. *This Journal*, lii, 209.
JOHNSON AND LUCKHARDT. 1927. *This Journal*, lxxxiii, 647.
KING, GARREY AND BLAIR. 1931. *This Journal*, xcvii, 329.
LENNOX AND COBB. 1928. *Medicine*, vii, 105.

PORTER. 1912. This Journal, xxxi, 223.

ROSETT. 1924. Brain, xlvii, 293.

SHERRINGTON. 1910. Quart. Journ. Exper. Physiol., iii, 213.

VAN SLYKE AND NEILL. 1924. Journ. Biol. Chem., lxi, 523.

WALSHE. Arch. Neurol. and Psychiat., x, 1.

WINKLER. 1929. This Journal, lxxxix, 243.

THE NUTRITIVE VALUE AND EFFICIENCY OF MINERALIZED MILK¹

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A discussion of the nutritive value of milk immediately brings to mind such common phrases as "milk, the perfect food" and "milk, the indispensable food." These statements are based on our knowledge concerning the general welfare of suckling mammals and upon certain observations made in the laboratory. The young of practically all mammals flourish reasonably well during the suckling period when milk is the sole source of food. When experimental animals are fed rations deficient in one of the basic constituents such as protein or one of the vitamins, milk in most cases has been found to supply this deficiency remarkably well. In fact the early experiments by Hopkins (1912) in which he obtained such extraordinarily good results when small amounts of milk were added to a diet of purified foodstuffs gave the real impetus to our modern studies on nutrition. Mann (1926) studying the importance of milk in the diet for boys between the ages of 6 and 11 years demonstrated that the group receiving one pint of milk in addition to a regular well balanced diet grew and developed much better than did the group not receiving the milk.

In spite of this exceedingly favorable attitude toward the nutritive value of milk, no one has been able, as far as we know, to rear a mammal from weaning to maturity on whole cow's milk alone. When rats are restricted after weaning to a diet of milk they grow for 3 to 4 weeks, reaching a weight of about 100 grams after which the animals decline in weight and die of anemia. The anemia which develops under these conditions has prevented the use of an exclusive milk diet for studying its nutritive properties. The inability of milk to produce hemoglobin was always attributed to its low iron content but Hart, Steenbock, Waddell and Elvehjem (1928) demonstrated that milk is also deficient in copper. This element acts as a supplement to iron in hemoglobin formation, and it must be added to milk as well as inorganic iron in order to prevent the development of anemia. When milk supplemented with iron and copper is fed to rats at

¹ Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

weaning fair growth is obtained, or if it is given to rats already suffering from anemia, the rate of growth increases with the improvement in the hemoglobin of the blood. In either case the rate of growth is not optimum. Curves given by Krauss (1931) show that male rats fed milk supplemented with iron and copper from weaning required almost 15 weeks to grow from 60 to 200 grams. It is more difficult to follow the growth records of rats which were first made anemic and then cured with iron and copper because the animals were often discarded as soon as the hemoglobin regeneration was complete, but the curves given by Waddell, Steenbock, Elvehjem and Hart (1929) show that the rats rarely gained more than 100 grams in a period of 5 weeks.

More recently Kemmerer, Elvehjem and Hart (1931), working with mice, and Skinner, Peterson and Steenbock (1932), working with rats, have demonstrated that the addition of traces of manganese to a diet of whole cow's milk supplemented with iron and copper has a favorable effect upon growth. Since the iron, copper, and manganese can be added as inorganic salts, milk mineralized with these salts should give an excellent diet for studying the nutritive value and efficiency of milk. We are including in this paper the results obtained with rats and pigs when fed such a ration.

EXPERIMENTAL. The rats were reared according to the technique outlined by Elvehjem and Kemmerer (1931). When the young were 21 days old the male rats in each litter were placed in individual cages equipped with wire screen floors and they were fed only whole milk mineralized with iron, copper, and manganese. These elements were added as solutions of FeCl_3 , CuSO_4 , and MnCl_2 so that 30 cc. of milk contained 0.5 mgm. Fe, 0.05 mgm. Cu, and 0.04 mgm. Mn. Fresh whole cow's milk (Holstein) was supplemented with these solutions each morning and fed ad libitum to the rats immediately. A sufficient supply was placed in a refrigerator to give the rats an additional supply in the afternoon. The consumption was determined by measuring the amount of milk fed daily and the amount remaining at the end of each day.

In order to have comparable growth records for rats reared on an ordinary diet several litters were reared on shavings. When the young were 21 days old, the males were placed in individual cages on shavings and continued on the stock ration and whole milk. The rats were weighed once weekly. The weighings were made at the same time of day each week.

In table 1 are tabulated the number of days required to grow from 60 to 200 grams and the average daily gain for a few typical rats raised on each of the two rations. It is readily seen that the growth of the rats on the mineralized milk was exceedingly good and that they made equally as rapid gains as those fed on the stock ration. An average daily gain of almost 4.0 grams is exceptional compared to Donaldson's (1924) standards

which give an average daily gain of 1.8 grams for male rats during a similar period of life.

The average of 36 days required for the rats to grow from 60 to 200 grams is almost identical with the results obtained by Bryan and Gaiser (1932) for rats grown on Mendel's special diet composed of yellow corn, skim milk powder, casein, and salts, together with liver and lettuce. The average daily gain of 3.9 grams on mineralized milk does not compare with the rapid gain of 5.3 grams obtained by Mendel and Cannon (1927) and the phenomenal growth of 6.1 grams recently obtained by Anderson and Smith (1932). However, both of these results were obtained with the rats used at the Connecticut Agricultural Experiment Station. Undoubtedly the strain of rats used in that laboratory is much larger than those used in our

TABLE 1
Rate of growth of male rats on mineralized milk and on the stock ration

MINERALIZED MILK			STOCK RATION		
Rat no.	Time required to grow from 60-200 grams	Average daily gain	Rat no.	Time required to grow from 60-200 grams	Average daily gain
	<i>days</i>	<i>grams</i>		<i>days</i>	<i>grams</i>
133	35	4.0	400	38	3.7
136	36	3.9	401	38	3.7
173	36	3.9	402	31	4.5
174	38	3.7	403	36	3.9
203	37	3.8	404	40	3.5
334	33	4.2	405	34	4.1
Average....	36	3.9	Average...	36	3.9

laboratory because Anderson and Smith speak of males weighing over 700 grams while the males in our colony rarely reach 500 grams.

The efficiency of the mineralized milk was also found to be quite high. An average for a considerable number of rats shows that only 2.25 grams of milk solids were necessary to produce 1 gram of gain in weight. Although these results are of a preliminary nature, they do substantiate in a very definite way the conclusions which have been based previously on indirect evidence.

A similar experiment was conducted with pigs. Ten young Yorkshire pigs were selected from the general herd shortly after weaning and placed in individual pens in the University swine barns. The floors were of concrete and shavings were used for litter. We wish to emphasize, therefore, that the pigs were not kept under as restricted conditions as were the rats. The whole cow's milk fed was limited so that all pigs consumed daily the same amount. During the first 4 weeks each pig consumed 12

pounds of milk per day, during the second four weeks, 18 pounds, the third 22, the fourth 24, and during the fifth and sixth four week periods the consumption was 30 pounds per pig daily. A solution containing 50 mgm. Fe as FeCl_3 , 5 mgm. Cu as CuSO_4 , and 5 mgm. Mn as MnCl_2 was added to the milk of each pig every morning. Twenty-five cubic centimeters of cod liver oil were given once weekly to insure a sufficient supply of vitamin D. The pigs were weighed every 4 weeks.

TABLE 2
Growth of pigs on mineralized milk

WEEKS ON DIET	WEIGHT OF PIGS				
	No. 6	No. 7	No. 8	No. 9	No. 10
	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
0	51	57	45	26	29
4	78	76	67	49	53
8	118	114	96	86	95
12	160	155	141	129	144
16	198	193	175	167	179
21	243	246	226	222	241
25	280	280	278	266	283

TABLE 3
Average gain of pigs on mineralized milk and a standard dry ration

WEEKS ON RATION	AVERAGE GAIN IN WEIGHT	
	Mineralized milk	Standard ration
	<i>lbs.</i>	<i>lbs.</i>
4	23.0	22.5
8	60.2	54.8
12	104.2	97.0
16	141.8	148.0

The pigs grew extremely well on this diet and appeared to be normal in every way. This diet apparently was very satisfying since the pigs appeared to be quiet and contented. The growth records of 5 typical animals are recorded in table 2. In table 3 the average gain in weight is compared with similar values obtained for a large group of pigs raised on a standard mixed ration consisting of yellow corn 77 parts, dried butter milk 14.5 parts, linseed meal 3 parts, alfalfa meal 5 parts, and iodized salt 0.5 part. Growth on the two rations is very similar. In fact during the first 12 weeks the pigs on the mineralized milk grew slightly faster than those on the dry ration. The pigs on mineralized milk have been raised

to maturity and reproduction is being studied at the present time. These results will be presented later.

The average daily gain of the pigs on mineralized milk during the first 16 weeks was 1.26 pounds. The gain is considerably greater than the average figure of 0.9 pound given by Henry and Morrison (*Feeds and Feeding*). The rate of gain would possibly have been greater had ad libitum feeding been practiced.

Upon calculating the efficiency of the milk ration and the dry ration, it was found that only 1.97 pounds of milk solids were necessary to produce 1 pound of gain, while 3.53 pounds of the standard ration were required for the same gain. These results demonstrate further the excellent nutritive value of milk alone when specific inorganic deficiencies in milk are corrected.

DISCUSSION. The direct feeding of mineralized milk to experimental animals will undoubtedly inaugurate many new problems. We know that iron and copper do not need to be added to milk for very young animals because there is a sufficient supply of these elements in the liver to counteract the deficiency in milk. The supply, however, varies in different species and in different animals of the same species. It is important to determine when the inorganic supplements should be added in order to give the best results. The destructive action of these elements on certain organic factors in the milk is another question which must be given consideration. The rapid growth resulting from the feeding of mineralized milk increases the requirement of other nutritive factors in the milk. If these factors are present in low amounts they may limit growth and development.

If the mineralization of milk is to be used under practical conditions the simplest and most efficient method of supplying these elements must be worked out. These are problems for the future, and in this paper we are merely demonstrating the possible importance of mineralized milk in experimental work.

SUMMARY

Rats reared from weaning on whole cow's milk mineralized with iron, copper, and manganese grew from 60 to 200 grams in 36 days. The average daily gain of 3.9 grams was very similar to the gain made by rats on an ordinary ration. Two and twenty-five hundredths grams of milk solids were required to produce a gain of 1 gram in weight.

Pigs reared on mineralized milk (plus cod liver oil) made an average daily gain of 1.26 pounds over a period of 16 weeks. The rate of gain was practically identical with that made by pigs on a standard dry ration. Only 1.97 pounds of milk solids were necessary to produce 1 pound gain in weight while 3.53 pounds of the dry ration were needed to produce the same gain.

The nutritive value and efficiency of whole cow's milk has been demonstrated by direct feeding after the deficiencies in the milk were corrected by the addition of inorganic supplements.

BIBLIOGRAPHY

- HOPKINS. 1912. *Journ. Physiol.*, xlv, 425.
MANN. 1926. Special Report Series no. 105, Medical Research Committee, London.
HART, STEENBOCK, WADDELL AND ELVEHJEM. 1928. *Journ. Biol. Chem.*, lxxvii, 797.
KRAUSS. 1931. Bull. 477, Ohio Agric. Exper. Sta.
WADDELL, STEENBOCK, ELVEHJEM AND HART. 1929. *Journ. Biol. Chem.*, lxxxiii, 251.
KEMMERER, ELVEHJEM AND HART. 1931. *Journ. Biol. Chem.*, xcii, 623.
SKINNER, Peterson AND STEENBOCK. 1932. *Biochemische Zeit.* CCL. 392.
ELVEHJEM AND KEMMERER. 1931. *Journ. Biol. Chem.*, xciii, 189.
DONALDSON. 1924. *The rat*. Philadelphia.
BRYAN AND GAISER. 1932. *This Journal*, xcix, 379.
MENDEL AND CANNON. 1927. *Journ. Biol. Chem.*, lxxv, 779.
ANDERSON AND SMITH. 1932. *This Journal*, c, 511.

THE NATURE OF THE FOODSTUFFS OXIDIZED TO PROVIDE ENERGY IN MUSCULAR EXERCISE

IV. THE USE OF PROTEIN AS A FUEL IN EXERCISE

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In previous papers of this series (Rapport and Ralli, 1928) evidence was brought forward to indicate that carbohydrate was not the exclusive source of oxidative energy in muscular contraction, but that muscles could utilize either carbohydrate, or fat, or both, depending upon the proportions in which these substances are presented to them in available form. No account was taken of the possibility that protein might also be used for this purpose.

Such opinion as exists on this point is based upon studies made upon the nitrogen metabolism, and particularly upon the presence or absence of a rise in the nitrogen excretion during and following muscular effort. It is well known (Pflüger, 1891) that the dog can exist on an almost purely protein diet, and there is inferential evidence that in the later stages of fasting, protein may contribute to the energy requirement of exercise.¹ Under ordinary conditions, however, evidence for the extensive utilization of protein in exercise is slight. On the question of whether muscular work is associated with a rise in nitrogen excretion opinion is divided. It is clear, however, that even with a large amount of work, the excreted nitrogen is at least not greatly increased, except perhaps after long continued starvation, and apart from this, nothing in this type of evidence indicates that a noteworthy percentage of the needed oxidative energy of muscular metabolism is derived from protein breakdown. This phase of the matter has been adequately reviewed by Cathcart (1925) and by Lusk (1928).

Nevertheless it seems to us that in a sense the study of the nitrogen excretion is somewhat irrelevant to the question of the utilization of protein energy. If one assumes that the nitrogenous fragment of deamination can not be used as a source of oxidative energy, then the matter

¹ In a paper reported by Dann at the meeting of the American Physiological Society, April, 1932, it was shown that the pre-mortal metabolism of a fasting dog was practically entirely protein, judging both from the nitrogen excretion and the respiratory exchange.

reduces itself to the utilization of the non-nitrogenous fragments, and studies of the nitrogen metabolism do not necessarily furnish evidence in regard to this utilization. For example, let us suppose that on an ample mixed diet with the subject at rest, the N excretion is 12 grams per 24 hours, indicating the apparent metabolism of 75 grams of protein. Let us further assume that with a heavy day's work the nitrogen excretion on the same diet remains at 12 grams. This proves no more than that. It may not even, as Cathcart has pointed out, prove that deamination has been no greater, providing protein synthesis has simultaneously occurred. Waiving this point, however, it certainly throws no light upon the fate of the non-nitrogenous portion, in the sense of ruling out the use of the latter to provide energy for the muscular exercise on the day of work.

This question we have studied, using the excess respiratory quotient of exercise as a criterion of the non-nitrogenous metabolism. The results indicate that protein, as well as carbohydrate and fat, can be used as a fuel in muscular exercise.

EXPERIMENTAL PROCEDURE. The experiments were performed on a bitch weighing about eleven kilos, the respiratory metabolism being obtained by the use of a closed system of the Benedict-Homans type previously described in communications from this laboratory. Exercise was accomplished by causing the dog to run on a horizontal treadmill introduced into the animal chamber, as described in a previous paper (Rapport, 1929). The exercise was mild, consisting of 15 minutes' running at approximately 2.5 kilometres per hour. With this degree of exertion recovery, as evidenced by the R. Q. and the oxygen consumption, was usually complete within $\frac{1}{2}$ hour. The experiment was ordinarily continued for 2 or 3 half-hour periods after this (longer if necessary), to establish the post-recovery resting level. The excess metabolism of exercise and recovery was calculated upon the difference between the gaseous exchange of exercise and recovery, and that of the average of the pre- and post-exercise resting periods. In a few instances this calculation was also made upon the pre-exercise period alone, when a possibility of difference in the interpretation of results made this desirable.

Our general method was to establish as nearly as possible an exclusive protein metabolism, and then to see whether during exercise the animal still continued to use protein as at rest. Our criterion of the amount of protein metabolism was the respiratory quotient, and since the quotient of a mixture of carbohydrate and fat can approximate that of protein, it was necessary for us first to create a one-sided metabolism of one or the other of these non-protein substances, in order to be able to differentiate this from the ensuing metabolism after giving protein. We attempted, therefore, to do the following: 1, to approach a theoretical respiratory quotient of 0.71 by fat feeding or starvation on days preceding the experi-

ment, or to approach a theoretical quotient of 1.00 similarly by carbohydrate feeding; 2, to give an amount of lean beef heart on the experimental day that would result in an exclusive or nearly exclusive protein metabolism at rest, as shown by a shift of the R. Q. to that of protein; 3, then to exercise the animal and see whether the R. Q. of exercise and recovery remained that of protein, or reverted to the prevailing quotient before the meat feeding.

The average nitrogen excretion per hour of the experimental period was determined and has been recorded. It must be emphasized, however, that this does not accurately represent the actual protein metabolism of the period, because of the delay in urea formation and excretion. As was shown in a previous paper (Ralli, Canzanelli and Rapport, 1931, 337), the nitrogen excretion may represent 50 per cent or less of the actual protein metabolism in an acute experiment after protein feeding, and this discrepancy is even more likely to occur in the short experiments reported in the present paper.

Four alcohol checks were performed during the course of the work, the individual respiratory quotients being 0.660, 0.656, 0.654, and 0.667, an average of 0.659.

EXPERIMENTAL RESULTS. *Protein feeding (a) after starvation and fat diet.* The results are summarized in table 1. After 48 hours of starvation (excepting 300 grams beef heart on the preceding day) the average resting R. Q. on October 8 was 0.74 and the excess R. Q. of exercise and recovery was the same. On the following day, after 24 hours more of starvation, 500 grams of lean beef heart were given, resulting in a resting R. Q. of 0.76 and an excess R. Q. in exercise and recovery of 0.79.

On October 10 and 11 a high fat diet, (including enough protein to maintain N equilibrium) was given, and on October 12 500 grams of beef were again administered. The resulting resting quotient before exercise was 0.79, which remained the same during exercise and recovery; but dropped to an average of 0.75 for the 4 half-hour periods following recovery. Calculated on the average resting metabolism before and after exercise, the excess quotient of exercise was 0.82. It would probably be more accurate to take the calculation based on the pre-exercise period, giving an excess R. Q. of 0.79, for it seems probable that in the later periods (4 to 5 hours after this meat feeding) the effect of the protein had begun to wear off. In either case, the evidence would indicate protein consumption during the exercise.

On the next day, October 13, the post-absorptive metabolism (no other food having been given) showed an R. Q. of 0.72, or practically that of fat. On giving 500 grams of beef, the resting R. Q. jumped to 0.79, indicating an almost exclusive protein metabolism, while that of exercise and recovery was 0.81, the excess R. Q. being 0.86.

TABLE 1
*Excess R. Q. of exercise and recovery*¹

DATE	MAINTENANCE RATION	EXPERIMENTAL DIET (BEFORE EXPERIMENT)	N EXCRETION PER HOUR	RESTING METABOLISM				EXERCISE AND RECOVERY	EXCESS R. Q.	
				Before exercise		After recovery	Average resting		Over pre-exercise resting	Over average resting
				Post absorp-tive	After food					
1931			gram							
Oct. 6	High carbohydrate* Oct. 5	None	0.078		0.93	0.89	0.91	0.91		0.90
Oct. 8	300 grams beef Oct. 7	None	0.110		0.74	0.74	0.74	0.74		0.74
Oct. 9	No food since Oct. 7	500 grams beef	0.339†		0.77	0.76	0.76	0.78		0.79
Oct. 12	High fat‡ Oct. 10, 11	500 grams beef	0.405		0.79	0.75	0.77	0.79	0.79	0.82
Oct. 13	High fat † Oct. 10, 11; beef experiment Oct. 12	500 grams beef	0.367	0.72	0.79	0.77	0.78	0.81		0.86
Oct. 14	Beef experiment Oct. 13	None	0.209		0.76	0.73	0.75	0.77	0.78	0.83
Oct. 15	No food since Oct. 13	None	Lost		0.73	0.74	0.73	0.73		0.72
Oct. 16	High fat‡ Oct. 15	500 grams beef	0.307	0.73	0.79	0.76	0.77	0.78	0.76	0.78
Oct. 19	High carbohydrate* Oct. 17, 18	500 grams beef	0.356	0.92	0.87	0.86	0.87	0.87		0.88
Oct. 20	High carbohydrate* Oct. 17, 18, 19	500 grams beef	0.318	0.92	0.85	0.89	0.87	0.88		0.88
Oct. 21	Carbohydrate diet Oct. 20 (55 grams cracker meal)	50 grams glucose	0.166		1.03	1.00	1.01	1.01		1.00
Oct. 22	25 grams glucose; 100 grams beef, Oct. 21	25 grams glucose; 100 grams beef	0.228		0.94	0.91	0.93	0.92		0.90
Oct. 23	25 grams glucose; 100 grams beef, Oct. 22	25 grams glucose; 100 grams beef	0.224		0.89	0.87	0.88	0.89		0.89
Oct. 24	25 grams glucose; 100 grams beef, Oct. 23	25 grams glucose	0.121		0.95	0.96	0.96	0.98		1.01
Oct. 27	High carbohydrate* Oct., 24, 25, 26	None	0.092		0.91	0.90	0.90	0.90		0.90

* 110 grams cracker meal, 10 grams glucose, and 40 grams beef heart.

† 40 grams lard and 40 grams beef heart.

‡ All determinations of nitrogen are the average of the experimental period, and consequently are not accurate indices, in protein feeding experiments, of the true protein metabolism.

On October 14 no food was given, but the effect of the high protein feeding was still manifest during the early hours, for the pre-exercise resting quotient was 0.76 and that of exercise 0.77, later dropping to 0.73 at rest (the N excretion was also still elevated). However, after another

TABLE 2
Energy exchange at rest and in exercise

DATE	EXPERIMENTAL DIET	RESTING METAB- OLISM (PER HOUR)	EXCESS METAB- OLISM (OVER BASAL)*	WORK†	CALORIES TO MOVE 1 KG. 1 METER	IN- CREASE OVER POST- ABSORP- TIVE EXCESS	SPECIFIC DYNAMIC ACTION
1931		calories	calories	kgm.		per cent	per cent
Oct. 6	Basal	14.0	7.1	7,340	0.97		
Oct. 8		14.4	9.0	7,145	1.26		
Oct. 14		16.2	6.5	6,698	0.97		
Oct. 15		15.8	6.9	6,578	1.05		
Average.....		15.2	7.4	6,940	1.06 (1.00)‡		
Oct. 9	Beef, 500 grams	19.4	10.8	7,016	1.54		28
Oct. 12		21.2	10.0	6,726	1.49		39
Oct. 13		20.6	9.3	6,698	1.39		36
Oct. 16		20.8	9.8	6,698	1.46		37
Oct. 19		22.6	10.6	7,002	1.51		49
Oct. 20		23.0	12.1	7,298	1.66		51
Average.....		21.2	10.4	6,906	1.51	45 (51)	40
Oct. 21	Glucose, 50 grams	19.8	8.5	7,032	1.10		30
Oct. 24	Glucose, 25 grams	18.6	7.6	7,556	1.01		22
Average.....		19.2	8.1	7,294	1.06	0 (6)	26
Oct. 22	Glucose, 25 grams; beef, 100 grams	20.4	8.5	7,032	1.10		34
Oct. 23		20.2	8.3	6,834	1.22		33
Average.....		20.3	8.4	6,933	1.16	12 (16)	34

* Not over resting metabolism of the experiment itself except in "basal" group.

† Loosely used to represent weight moved through distance horizontally.

‡ Excluding October 8.

day of starvation both the resting R. Q. and that of exercise had fallen to the normal fasting levels (0.73 and 0.72, respectively). After the experiment, on this day, a high fat diet was again given, and on the following day the post-absorptive quotient was still 0.73. Upon giving 500 grams of beef, the R. Q. jumped to 0.79 before exercise and 0.78 during exercise and recovery; falling later to 0.76 as the protein effect wore off.

From the above experiments, it seems evident that whereas on fat feeding or during starvation the animal used fat as a source of oxidative energy both at rest and during exercise, confirming previous observations (Rapport and Ralli, 1928) upon the ingestion of protein the animal not only oxidized protein during rest, but also during exercise, for the rise in quotient was large and unmistakably beyond any error in the method. The R. Q. being approximately the normal R. Q. of meat protein, we must conclude that both the sugar forming and non sugar-forming amino acids were oxidized.

In table 2 will be seen data on the energy exchange, which show that it required 45 per cent more energy to move 1 kilo 1 meter after 500 grams of meat had been fed than in the post-absorptive state, and there is no doubt, as originally shown by Rubner (1910) and confirmed by Anderson and Lusk (1917) and Rapport (1929), that the specific dynamic action of the protein was not abolished during the exercise. It is reasonable to suppose, though admittedly not absolutely proved, that in these experiments, the split-products of amino acid metabolism were oxidized for energy in exercise without having been first converted to glucose and fat. If this is true, it would throw doubt on theories which postulate that the specific dynamic action of protein is due to waste energy involved in the conversion to glucose.

Protein feeding. (b) After carbohydrate diet. This phase of the work requires a few words only. Reference to experiments 1, 10, 11, and 15 in table 1 will show that these experiments were not conclusive. In the first place we were unable to obtain an exclusive carbohydrate metabolism, the post absorptive R. Q. being never above 0.92. In the second place, in the presence of the carbohydrate plethora, it is of interest that we could not produce an exclusive protein metabolism on giving meat, the resting quotient not going below 0.87. It is difficult to say what percentage of protein metabolism this represents; if protein were substituted for fat metabolism, the maximum protein metabolism would have been about 60 per cent, but it would not be safe to make the assumption that this is what actually happened. Such change as occurred in the resting quotient, however, was reflected in the exercise quotient, and, as far as they go, the experiments were confirmatory of those after fat ingestion.

Mixed protein and carbohydrate feeding. We wished to see whether the type of food used in exercise would be different if carbohydrate alone were given or a mixture of carbohydrate and protein. On October 21, 50 grams of glucose were administered with resulting resting and exercise quotients of 1.01 and 1.00 respectively. On the following day a mixture of 25 grams of glucose and 100 grams of beef heart, having a theoretical respiratory quotient of 0.90, was given. The average resting R. Q. was 0.93; the excess R. Q. of exercise and recovery 0.90. The next day the experiment

was repeated, and again the excess quotient of exercise (0.89) was practically the theoretical quotient of the mixture, although 25 grams of glucose alone contained more than enough energy to take care of the exercise. Giving this amount of glucose alone on the next day resulted in an excess quotient of 1.01, showing exclusive use of sugar in the exercise.

These experiments showed that in the presence of a plethora of both sugar and protein each could be used in exercise without apparent preference for either.

Extending the conclusions of previous work, it appears that in muscular exercise, the oxidative energy can be supplied not only by carbohydrate and fat, but by protein as well, and apparently quite as readily by the latter as by the other two major foodstuffs.

CONCLUSION

Protein is a normal source of oxidative energy for muscular exercise, and its non-nitrogenous split products are as readily available for this purpose as are carbohydrate and fat.

BIBLIOGRAPHY

- ANDERSON, R. J. AND G. LUSK. 1917. *Journ. Biol. Chem.*, xxxii, 421.
CATHCART, E. P. 1925. *Physiol. Rev.*, v, 225.
LUSK, G. 1928. *The science of nutrition*, 4th ed.
PFLÜGER, E. 1891. *Pflüger's Arch.*, 1, 98.
RALLI, E. P., A. CANZANELLI AND D. RAPPORT. 1931. *This Journal*, xcvi, 331.
RAPPORT, D. AND E. P. RALLI. 1928. *This Journal*, lxxxiii, 450.
RAPPORT, D. 1929. *This Journal*, xci, 238.
RUBNER, M. 1910. *Sitzungsber. d. preuss. Akad. d. Wissensch.*, xvi, 316.

SYMPATHIN AND THE HEPATIC SYMPATHOMIMETIC HORMONE IN THE DOG

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Previous work on sympathin (Newton, Zwemer and Cannon, 1931; Cannon and Bacq, 1931; Rosenblueth and Schlossberg, 1931; Rosenblueth and Cannon, 1932) and on the adrenin-like substance liberated from the liver by stimulation of its sympathetic supply (Cannon and Uridil, 1921; Cannon and Griffith, 1922; Rosenblueth and Cannon, 1932) has been performed mainly on cats. The only exception is Bacq's (1931) work on the iris of the rabbit. It was considered of interest to learn whether similar phenomena would be presented by other species, thus giving a more general character to the conclusions derived. After some trials on the heart and blood pressure of the rabbit the dog was chosen because of greater ease in applying the techniques involved.

METHOD. The responses of the denervated heart were studied either by using the method employed by Cannon and Bacq (*loc. cit.*) or by placing the animal under light dial anesthesia. The changes of blood pressure were investigated with the preparation described by Rosenblueth (1932).

The heart was sensitized by denervation at least 5 days before. The sensitization of the blood-pressure response by cocaine was generally increased by removal of the stellate, semilunar, and superior and inferior mesenteric ganglia, again at least 5 days before.

The adrenals were commonly ligated or otherwise inactivated, either acutely or several days before the experiment.

Heart rates and blood pressures were recorded through femoral or carotid arterial cannulae connected to a mercury manometer. Stimulation of the lower abdominal sympathetic chains (l.a.s.) at about the level of L3 and of the nerves surrounding the duodeno-hepatic artery (h.n.), after both groups of nerves had been centrally crushed or cut, was effected by shielded electrodes in the secondary circuit of a Harvard inductorium. Tetanizing frequencies were used.

RESULTS. The responses of the above indicators differ only quantita-

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tively when either the l.a.s. or the h.n. are stimulated for 30 to 60 seconds. The responses are greater in the second case.

There is an increase of the rate of the denervated heart, which begins to be noticeable about one minute after the start of stimulation, though sooner for the h.n. than for the l.a.s. It reaches its maximum about 3 minutes later and lasts between 5 and 10 minutes. The increases observed in these experiments ranged from 10 to 30 beats per minute (approximately from 6 to 20 per cent). They could be repeatedly obtained. The highest rise in the rate of the heart coincided in time with the peak of the blood-pressure changes.

When the l.a.s. is stimulated there is an initial rise of blood pressure (see fig. 1), due undoubtedly to vasoconstriction. This persists during stimulation, producing a plateau. About one minute later, usually after the end of the stimulation, a second rise appears, bringing the blood pressure to a higher level. Finally there is a gradual return to normal. Sometimes both rises blend into a single curve, but there is invariably, for about two minutes after the stimulation has ceased, a continuing increase which cannot be accounted for by the vasoconstrictor effects of the nervous stimulation.

Stimulation of the h.n. yields similar but more marked effects. These persist when the hepatic artery is clamped.

If the circulation to the hind part of the body is occluded by pulling a thread passed around the aorta and the cava below the renal vessels there is a rise of blood pressure (see fig. 2-A). When the thread is released there is a sharp fall, followed by a slow return to the original level. If the l.a.s. are stimulated while the thread is being pulled (see fig. 2-B) the rise during the occlusion is followed by a fall on release, but the pressure does not go below the base line. There then occurs a new rise, usually higher than that resulting from pulling the thread, which, again, cannot be accounted for by the constrictor effects of the stimulation.

Emotional excitation of the sympathetic in the unanesthetized animal (Newton, Zwemer and Cannon, loc. cit.) increases also the rate of the denervated heart even when all nervous connections to the liver and adrenals have been removed. Complete sympathectomy abolishes this response of the heart. Similar results were observed by us in the dog.

SUMMARY

The responses of the denervated heart and of blood pressure to peripheral stimulation of the hepatic nerves and of the lower abdominal sympathetic chains were studied in the dog.

The results obtained (see figs. 1 and 2) are strictly comparable to those found in the cat in previous papers published from this laboratory.

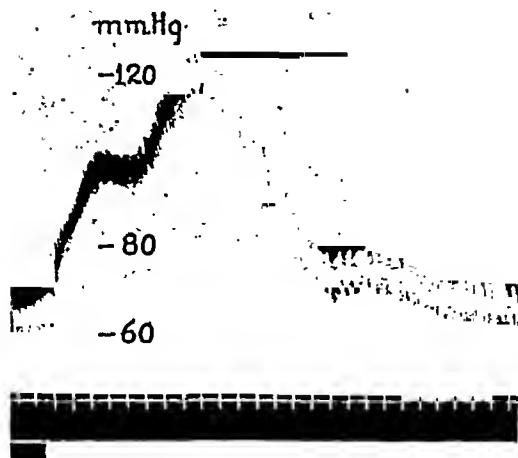


Fig. 1. Blood pressure response on one minute peripheral stimulation of the sectioned lower abdominal sympathetic chains at L3. Dial, 0.25 cc. per k. intravenously. Cocaine, 8 mgm. per k. intravenously. Vagi cut. Stellate ganglia and upper thoracic sympathetic chains removed 6 days previously. Spinal cord transected at D2 and adrenals inactivated the day preceding the experiment. Time intervals, 30 seconds.

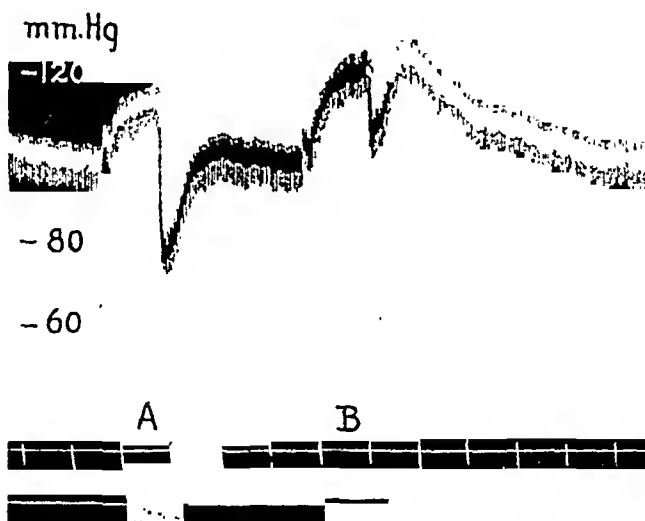


Fig. 2. Stellate, semilunar, and superior and inferior mesenteric ganglia removed 10 days before. Dial, 0.7 cc. per k. intraperitoneally. Vagi cut. Adrenals ligated. Heavy thread passed under the aorta and the vena cava immediately below the renal vessels. Time intervals, 1 minute.

A. Effects of occluding the circulation to the hind part of the animal 1 minute.

B. Similar occlusion with simultaneous excitation of the lower abdominal sympathetic chains. See text for interpretation of records.

Sympathin and the sympathomimetic substance liberated from the liver are, therefore, not limited to the feline species.

BIBLIOGRAPHY

- BACQ, Z. M. 1931. C. R. Soc. Biol., cvii, 1584.
CANNON, W. B. AND Z. M. BACQ. 1931. This Journal, xcvi, 392.
CANNON, W. B. AND F. R. GRIFFITH. 1922. Ibid., lx, 544.
CANNON, W. B. AND J. E. URIDIL. 1921. Ibid., lviii, 353.
NEWTON, H. F., R. L. ZWEMER AND W. B. CANNON. 1931. Ibid., xcvi, 377.
ROSENBLUETH, A. 1932. Ibid., ci, 149.
ROSENBLUETH, A. AND W. B. CANNON. 1932. Ibid., xcix, 398.
ROSENBLUETH, A. AND T. SCHLOSSBERG. 1931. Ibid., xcvii, 365.

STUDIES ON THE REGULATION OF WATER INTAKE

I. THE EFFECT OF EXTIRPATION OF THE SALIVARY GLANDS ON THE WATER INTAKE OF DOGS WHILE PANTING

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The close correlation between appearance of thirst and a marked reduction in the salivary flow observed by Cannon (1918) led him to conclude that thirst is really what the ordinary individual thinks it is, namely, a dryness of the mouth, and that it is brought about when the salivary glands are prevented from discharging sufficient fluid with which to moisten adequately the buccal mucosa. Montgomery (1931a and b), in experiments on dogs deprived of their salivary glands, or studied in relation to the effects of drugs (pilocarpine and atropine) on the water intake, has obtained results which tend to discredit this explanation of the thirst sensation and to minimize the importance of the salivary glands in relation to the water intake. She has offered no alternative explanation. The results are of a negative character.

The problem of the importance of the salivary glands in relation to thirst raises two crucial questions: 1. Can thirst, when caused by water deprivation, be alleviated by merely wetting the mouth? 2. Does thirst appear in the absence of bodily dehydration when mouth and throat become dry?

1. Can thirst, when caused by water deprivation, be alleviated by merely wetting the mouth? Common testimony indicates that there is an urgent desire for water before the subject is aware of general functional disturbances, due to need for it, and this is borne out by the fact that the subject may obtain relief from measures which do not increase the water content of the body. "Commonly the condition is alleviated by a moderate quantity of water; sometimes fruit acids and other sapid substances exciting flows of saliva are requisite for relief; and in the practical life of the range a pebble or nail carried in the mouth is often efficacious" (McGee, 1906). "When water is scant . . . it may be economized by a method well known in all arid regions—that of alleviating local dryness of the buccal and other membranes by sipping and sniffing a few drops at a time, and allowing the general condition to take care of itself. Many vaqueros and prospectors become artists in mouth moistening and carry

canteens only for this purpose (depending on lavish draughts at camp to supply the general needs of the system) . . . " (ibid.). From McGee's (1906) personal observations one cannot fail to be impressed with the fact that the thirsty man's chief concern is to moisten his mouth and be relieved of the tormenting dryness in his mouth and respiratory passages.

Some years ago Pack (1923) showed that the quantity of water drunk in one hour by rabbits which had been deprived of water for seven days was greatly reduced by an injection of pilocarpine (0.5 cc. of 1 per cent solution per kilogram body weight) given shortly before water was offered the animals. Pack concluded from these observations "that pilocarpine . . . relieves thirst by stimulating the flow of saliva." His experiments have been repeated in this laboratory (Gregersen and Bloomberg, unpublished) and the observations confirmed, but it was noticed that in every case the injected rabbits were more or less prostrated for some time after the drug had been given. Therefore, it cannot be said with certainty that these rabbits drank less than the controls solely because of an increased flow of saliva. It seems probable that the effect of the pilocarpine upon their general well-being caused them to drink less.

Montgomery (1931b) found that "injections of pilocarpin, after a two day thirst period, did not diminish the water intake of normal dogs as compared with that of dogs which had been deprived of their salivary glands," and, except in one case, the dogs which received pilocarpine drank more than the dogs which had not. These observations indicate that pilocarpine fails to relieve thirst by promoting a free flow of saliva and, therefore, disagree with Pack's experiments. We have repeatedly observed that any dose of pilocarpine which induces abundant flow of saliva also has a depressant effect on dogs. Indeed, Montgomery (1931b) frankly states that in several of her experiments the drug had an emetic action. If vomiting is accompanied by as much discomfort and malaise in the dog as in man, one can hardly regard a pilocarpinized animal as being in a favorable condition for showing whether or not an increased salivary flow can alleviate thirst. It would be advisable to study the relation between salivary flow and thirst without recourse to experimental procedures with such general and ill-understood bodily effects.

The question of whether or not thirst can be alleviated by merely wetting the mouth is therefore left unanswered by experiments involving the use of pilocarpine for exciting a flow of saliva, and we are left to draw conclusions from common experience.

2. Does thirst appear in the absence of bodily dehydration when mouth and throat become dry? "Breathing hot air free from moisture, prolonged speaking or singing, the repeated chewing of desiccated food, the inhibitory influence of fear and anxiety on salivary secretion, have all been observed to result in dryness of the buccal and pharyngeal mucous membrane and in

attendant thirst" (Cannon, 1918). Whether or not this form of thirst is exactly the same quality of sensation as the thirst produced by water deprivation, it is at least evident that the end result is the same, namely, the desire to drink, and it shows that local dryness of the mouth unaccompanied by bodily need for water may lead to increased water intake.

Montgomery (1931a) attempted "to make a crucial test of the importance of the salivary secretion in the control of water ingestion by completely extirpating the salivary glands." This investigator reasoned that "if . . . a diminished secretion of saliva leads to local dryness of the mouth, which in turn causes the animal to drink, a total elimination of the salivary secretion should produce an increase in the daily ingestion of water by the animal" (1931a).

After tying the salivary ducts in dogs, Bidder and Schmidt (1852) observed a remarkable decrease in the fluid wetting the mouth so that only at best was this sufficient for keeping the mucosa moist, when the mouth was held closed; ". . . bei erleichtertem Luftzutritt . . . ein wirkliches Trockenwerden" was scarcely prevented. The dogs had difficulty in swallowing not only dry food such as bread, but even raw meat. Thirst was greatly increased so that the dogs were always ready to drink. Unfortunately, Bidder and Schmidt gave no data on the water intake. In two dogs Fehr (1862) extirpated the salivary glands, including the infra-orbitals. After the immediate effects of the operation had worn off, the only change noticed was that the dogs then drank somewhat more water "probably to facilitate chewing and swallowing of food." Montgomery (1931a), on the other hand, found that the average daily water intake of dogs kept under laboratory conditions was not increased after the complete removal of submaxillary, sublingual, parotid and infraorbital glands. She concluded from her observations, "it appears improbable that the salivary glands play a major rôle in the thirst mechanism."

But this conclusion does not necessarily follow from her data. Her observations were made under conditions where a small secretion into the buccal cavity may have been adequate for keeping the mouth and tongue moist enough to prevent increased thirst. After extirpation of the salivary glands the dogs still possessed other glands which could supply fluid for wetting the buccal cavity. The secretion must have been adequate to prevent drying of the mucosa *under the conditions of her experiments*, for she speaks of "the remarkably moist condition of the mucous membrane of the mouth which can exist in the entire absence of the salivary glands." This agrees with observations we have made. In one of Montgomery's dogs, no. 7, the secretion poured out by the glands of the buccal, pharyngeal and nasal mucosa was collected from an esophageal fistula. The secretion measured 8.9 grams per hour when the dog was quiet, 1 gram per hour when the dog was asleep. This, of course, does not include water carried

off by the expired air. Removal of the salivary glands has obviously not eliminated all mechanisms for keeping the mouth wet.

Montgomery's dogs, moreover, were well supplied with water and were given a moistened food (25 grams of water per 100 grams of food). The activity of the remaining secretory tissue in the buccal cavity was therefore not depressed by bodily dehydration. It is evident that, although the salivary glands supply a watery secretion, they cannot be regarded as the sole

TABLE 1

Showing the effect on water intake before and after exclusion of the salivary flow when dogs were exposed to a temperature of 40°C.

DOG NUMBER	WEIGHT	WATER SUPPLIED BEFORE EXPERIMENT	DURATION OF EXPOSURE	BEFORE OPERATION			AFTER OPERATION			CHANGE IN WATER IN- TAKE	AFTER BEFORE
				Number of obser- vations	Water taken (aver- age)	Water taken	Number of obser- vations	Water taken (aver- age)	Water taken		
	kgm.		hours	days	cc.	cc. per kgm.	days	cc.	cc. per kgm.	cc.	
1	20.0	ad libitum	2	7	301	15.0	29	586	29.3	+285	1.95
2	15.0	ad libitum	2	9	447	30.0	19	417	27.8	-30	0.94
3	12.0	ad libitum	2	11	217	18.0	18	450	37.5	+233	2.07
4	13.5	ad libitum	2	9	231	17.0	23	518	38.4	+287	2.24
6	18-19 (18.5)	ad libitum	1	8	46	2.5	10	134	7.3	+88	2.9
		none for 6 hours	1	7	103	5.6	7	136	7.4	+33	1.32
		none for 18 hours	1	6	183	9.9	14	209	11.3	+26	1.14
7	13-15 (14.0)	ad libitum	1	8	195	13.9	12	287	20.5	+92	1.47
		none for 18 hours	1	5	350	25.0	17	513	36.6	+163	1.47
8	18.0	ad libitum	1	8	72.5	4.0	9	244	13.5	+171.5	3.36
		none for 18 hours	1	6	153	8.5	21	350	19.4	+197	2.29
9	21-19 (20.0)	ad libitum	1	8	135	6.8	9	72	3.6	-63	0.53
		none for 6 hours	1	7	127	6.4	9	105	5.3	-22	0.83
		none for 18 hours	1	6	263	13.2	15	190	8.5	-73	0.72

Dog 5 in this series was a young animal that died of distemper.

source of fluid wetting the mouth. On the other hand, the fact that *under favorable circumstances* the mucous glands may moisten the mouth sufficiently to obviate thirst does not rule out the importance of the salivary glands when there is greater need for fluid than the other glands of the mouth can supply.

To find what rôle the salivary glands actually play in the thirst mechanism, we believe that the effect of their extirpation upon water intake

should be tested under experimental conditions which lead to drying of the buccal mucosa. This can be accomplished by taking advantage of a special mechanism in the dog for lowering the body temperature. When exposed to a warm atmosphere the dog begins to pant. The mechanism is reflex and mediated by cutaneous sensory nerves (Sihler, 1879). The panting is accompanied by a flow of thin watery saliva (Welikanoff, 1928) which in this case has been definitely shown (Gregersen, 1931) to arise from dryness of the mouth and tongue and which helps to keep the mouth moist. In the absence of salivary glands, dryness of the mouth would be intensified since it can no longer be mitigated by a reflex secretion of

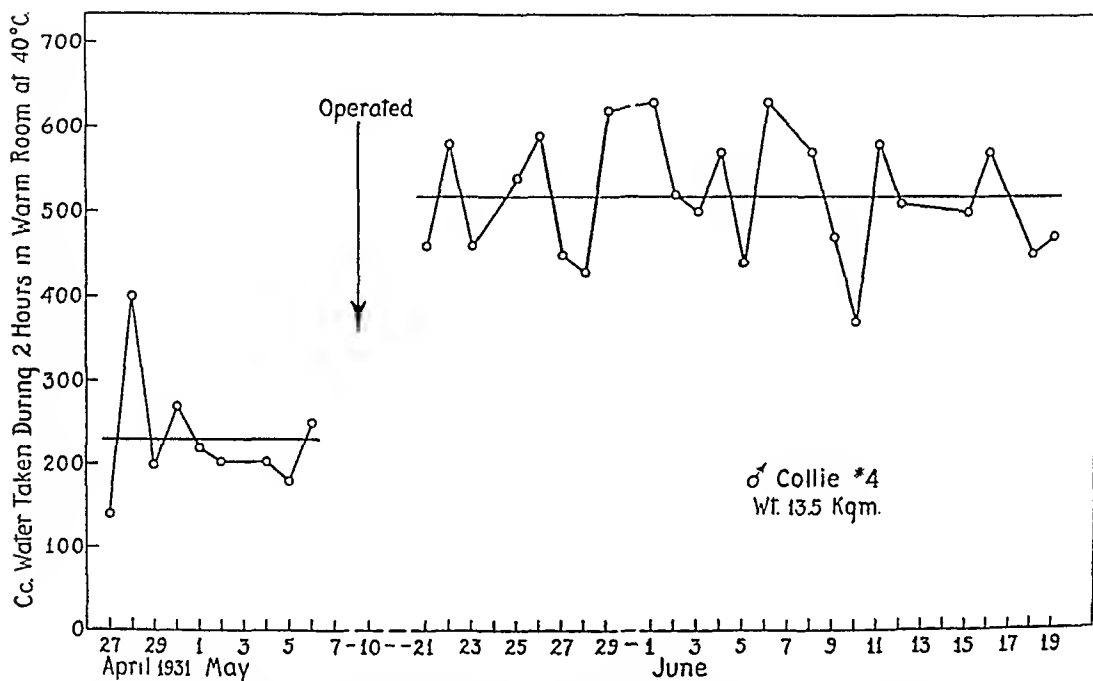


Fig. 1. Daily observations on dog 4, showing the amount of water taken during 2 hours in the warm room (40°C.) before and after exclusion of the salivary flow.

saliva. If thirst is caused by a dry mouth, the dog should drink more than normally.

EXPERIMENTS. We have attempted to investigate this question by measuring the water intake of dogs during periods of panting before and after exclusion of the salivary flow. Four dogs were used in the first series. They were kept in a warm room (40°C.) for 2 hours at approximately the same time each day, and the water drunk during the 2 hours was measured. At this temperature the panting was usually continuous, although dogs do not all respond equally well to a warm atmosphere. After readings had been made during a control period lasting on the average 10 days, the

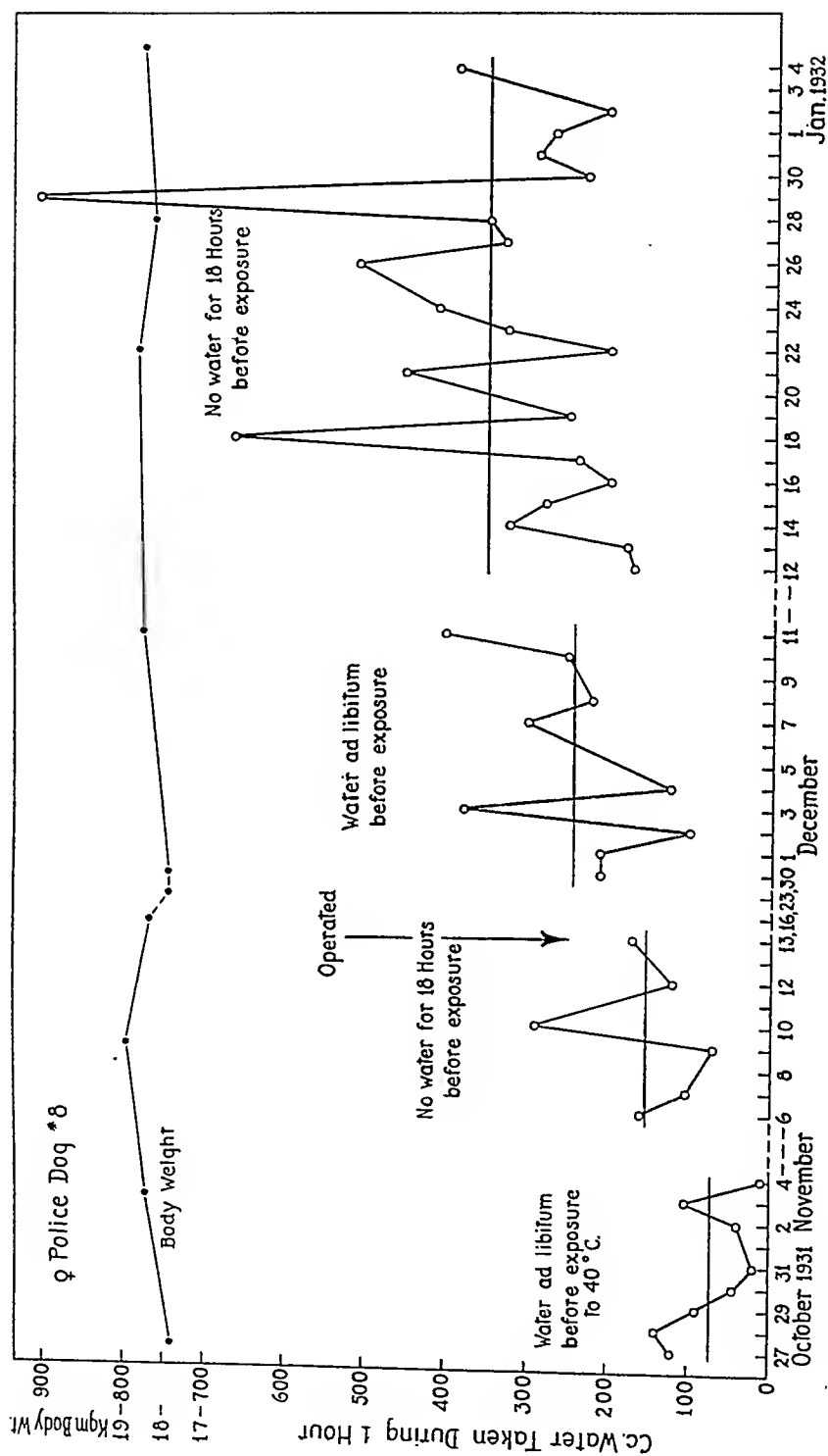


Fig. 2. Daily observations on dog 8, showing the effect of exclusion of the salivary flow on the water intake during 1 hour in the warm room (40°C.) when the dog had water *ad libitum* before entering and also when it had none for 18 hours before.

parotid ducts were tied and cut between ligatures, the submaxillary, sublingual, and infraorbital glands were extirpated, all in the same operation and with aseptic precautions. Recovery was complete in about 10 days, except in the case of dog 2 which required 20 days. The dogs were again placed in the warm room for 2 hours daily and the water intake during the 2 hours measured. Averaged results are shown in table 1 and the daily observations on one of the dogs (4) in figure 1.

In the second series (dogs 6, 7, 8 and 9), the daily exposure to 40°C. was limited to 1 hour. Tests were made not only when the dogs had been given water *ad libitum* up to the time of entering the warm room, but also when they had been deprived of water for 6 hours and for 18 hours before each test. The averaged results for these animals are also included in table 1 with those of the first series. The daily observations on one of the dogs (8) are shown in figure 2.

EXPERIMENTAL RESULTS. With the exception of dog 2, which was an old dog in rather poor condition, the animals of the first series drank approximately twice as much water during 2 hours in the warm room after the operation as before. When calculated in terms of centimeters of water taken per k. body weight, the water intake of dog 2 was much higher than in the others during the control period,—nearly as high as for the other dogs after the operation. It may be that the salivary flow of dog 2 was already deficient before the glands were removed. Autopsy failed to show anything to account for this exceptional result.

In the second series of animals we again find one dog (9) which failed to show an increase in the water intake after the operation. This dog suffered for over two weeks from an extensive subcutaneous necrosis in the groin and abdominal region, due to an infection. The lymph nodes in the groin were greatly enlarged, and each day for some time necrotic material was discharged through a hole in the skin. In two weeks the dog lost 3 k. in body weight. These complications, we believe, are sufficient reason for excluding the results obtained on this dog.

From the second series it may be seen that water deprivation for 6 hours or for 18 hours made surprisingly little difference in the water intake in the warm room. The animals were fed immediately after the daily tests and were given water for some hours after eating. In the following paper it will be shown that dogs (with or without salivary glands) drink very little during 24 hours except in the postprandial period. We can therefore understand why depriving the animals of water, except during 2 to 5 hours after feeding, causes an insignificant reduction in the 24-hour intake.

From the results of both series of experiments it appears that 6 of the 8 dogs drank more water in the warm room after the operation. This was true whether the dogs were deprived of water for some hours before the tests or were given water *ad libitum*. The last column in table 1 shows the

ratio of water intake after the operation to water intake before it, under the same experimental conditions.

As a rule, the dogs in the second series did not pant as vigorously or continuously in the warm room as the first series of animals, perhaps due partly to the shorter time of exposure. Dogs differ, however, in their response to heat and it may be that more marked results could be obtained if animals were chosen which pant consistently when exposed to high temperatures.

Autopsies on dogs 1, 2, 7 and 9 showed that the operations on them had been successful.

The same diet, consisting of ground cooked horse meat mixed with "Old Trusty All Terrier Food," was given throughout the experiments. The dogs took this mixture eagerly, although they were seen to eat more slowly after the operation.

SUMMARY AND CONCLUSION

The amount of water taken by dogs during 1 or 2 hours of panting is increased after extirpation of the submaxillary, sublingual and infra-orbital glands and tying the parotid ducts. Therefore, under conditions which make the mouth dry, a deficient salivary flow causes thirst and increased water intake even in the absence of bodily dehydration (see table 1, and figs. 1 and 2).

BIBLIOGRAPHY

- BIDDER, F. AND C. SCHMIDT. 1852. *Verdaugungssäfte und Stoffwechsel* (Leipzig), 3.
CANNON, W. B. 1918. *Proc. Roy. Soc., B*, xc, 283.
FEHR, C. 1862. *Arch. f. path. Anat.*, xxv, 186.
GREGERSEN, M. I. 1931. *This Journal*, xevii, 107.
MCGEE, W. J. 1906. *Interstate Med. Journ.*, xiii, 279.
MONTGOMERY, M. F. 1931a. *This Journal*, xevi, 221.
1931b. *Ibid.*, xeviii, 35.
PACK, G. T. 1923. *Ibid.*, lxxv, 346.
SIHLER, C. 1879. *Journ. Physiol.*, ii, 191.
WELIKANOFF, A. N. 1928. Reported by B. P. BABKIN. *Die Äussere Sekretion der Verdauungsdrüsen* (Berlin), 127.

STUDIES ON THE REGULATION OF WATER INTAKE

II. CONDITIONS AFFECTING THE DAILY WATER INTAKE OF DOGS AS REGISTERED CONTINUOUSLY BY A POTOMETER

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In carrying out a series of experiments on the rôle of the salivary glands in relation to the water intake of dogs (Gregersen and Cannon, 1932), I felt that records of their drinking habits were essential in order to be sure that the drinking was not affected by factors other than those we were deliberately imposing upon them. This led to the construction of a simple apparatus for obtaining continuous 24-hour records of the water intake. A study of these records sheds new light on the relation between digestion and drinking.

The potometer. A diagram of the essential parts of the apparatus may be found in figure 1. A reservoir, *R*, consisting of an airtight copper can of 1.5 liters capacity, is suspended from a coiled spring which shortens about 1 cm. for each 100 cc. of water taken from the can. It is connected by a rubber tube, *T*, supported by a wooden splint, to a short brass pipe, *P*, soldered to the side of a drinking pan, *DP*. Fluid leaves the reservoir whenever the water level in the pan falls below the outlet of the brass pipe, and this is registered by the shortening of the coiled spring. A writing point attached to the lower end of the spring records the emptying of the reservoir on a smoked drum which turns once in 12 hours. Removal of less than 25 cc. of water from the drinking pan is not recorded on the drum; therefore the record may not show the actual number of times water is taken.

The potometer is calibrated by pipetting 100 cc. at a time from the drinking pan and recording on the smoked drum the shortening of the spring. The graduated scale thus obtained enables one to read directly on the records the quantity of water taken by the dog over any given period of time. The calibration shows that the change in length of the spring upon decreasing the load 100 grams is about the same when the reservoir is nearly empty as when it is full. Therefore, no correction for changes in elasticity of the spring is necessary throughout the range of loads used here.

Correction for evaporation was not deemed essential since it amounted to less than 15 cc. per day.

The kymograph used was an old type which turns from left to right; therefore, in order to make the records read in the usual fashion they have been reversed. Also they have been turned upside down. Consequently, a downward stroke on the record signifies emptying of the reservoir.

EXPERIMENTS AND RESULTS. *Drinking records on normal dogs.* The drinking records shown in figure 2 are typical of those obtained on all normal dogs kept in cages in the laboratory. They live an inactive life, since their cages afford only limited space for moving about. Once a day

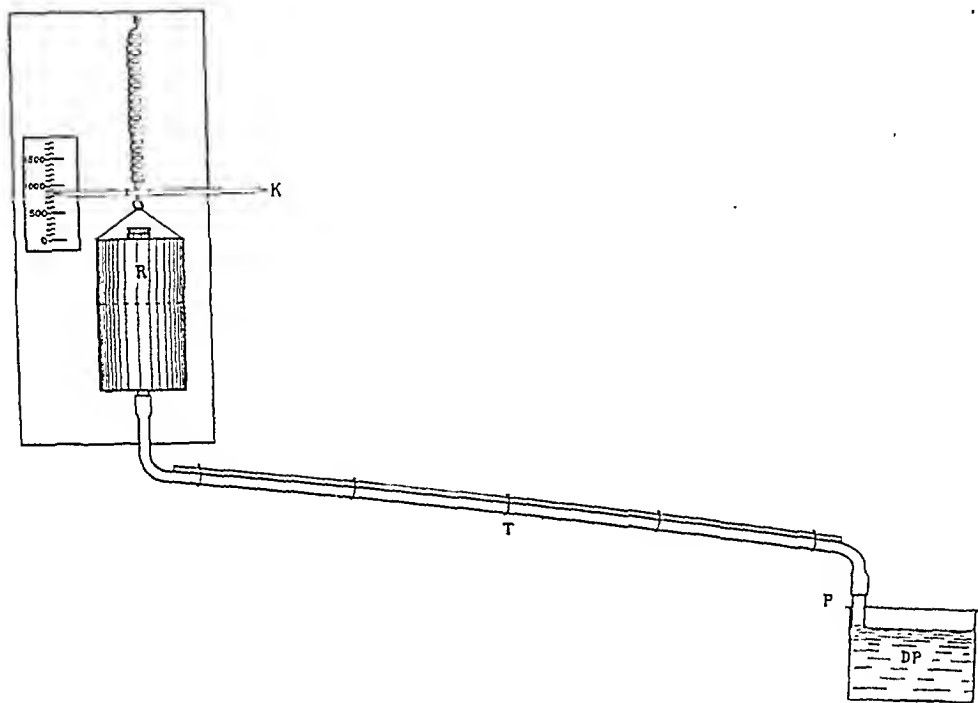


Fig. 1. The potometer. When the dog drinks from the pan *D P*, and the water level falls below the brass pipe *P*, air bubbles pass up the rubber tube *T* and release water from the reservoir *R*. The quantity of water taken is shown on the scale and is registered on a smoked drum by the writing point *K*.

they are fed a meal consisting of ground cooked horse meat mixed in about equal parts with "Old Trusty All Terrier Food," a crushed cracker.

The dogs all drink when they have eaten, rarely before the meal is finished, taking water from time to time during 2 to 5 hours after the meal; they drink very little until the next day when they are fed again (see records of "Mike" and dog 12, fig. 2). The drinking is evidently not a matter of habit associated with some particular time of day, for it always follows upon feeding no matter what time the food may be given (cf. records of dog 12 and "Mike," fig. 2). Moreover, the impulse to drink does not

arise solely, if at all, from the fact that the food may be dry and therefore hard to swallow, because the period of water intake extends over several hours after meals.

This increased desire for water postprandially, or "soif digestive," (Longet, 1868; Binet, 1931) naturally raises the question of what happens to the drinking when no food is given.

Drinking records on fasting dogs. Figure 3 shows the effect of fasting upon drinking. Number 22, a normal police dog of 18 k., drank only about 100 cc. in 2 days when food was withheld (see record, fig. 3, March 10 to 12). On March 12 this animal was fed and within 15 minutes it took its first drink; it consumed 600 to 700 cc. in 4 hours and a total of about

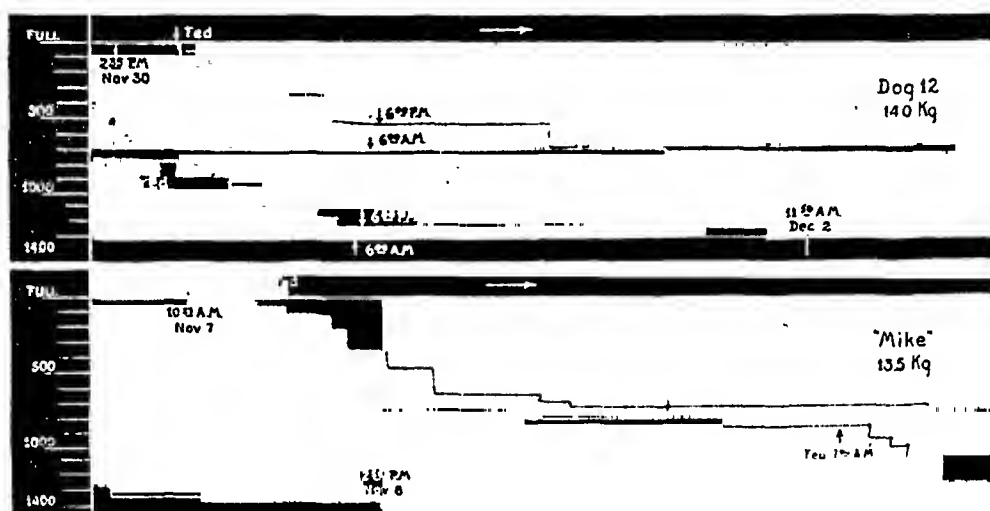


Fig. 2. Normal drinking records on dog 12 and "Mike," showing that most of the water drunk during 24 hours is taken within a few hours after the dogs are fed, no matter when the food may be given. In these and other drinking records the length of the record covers a period of 12 hours.

1000 cc. in 24 hours. "Mike," a terrier of 13.5 k., with double submaxillary fistulae of long standing, showed the same result less strikingly. During 48 hours of fasting, the dog drank in all 700 cc. On the third day he drank, within 5 hours after being fed, 750 cc.

These results are in agreement with observations made by Kleitman (1927). He found that starving dogs drink about 100 to 300 cc. of water per day, while the normal water intake, including water in food, is about 500 to 700 cc. per day. Kleitman (p. 339) suggests, merely as a possibility, that "the decrease in total metabolism, entailing a diminution in excretion of nitrogenous waste products and other crystalloids by the kidneys, with consequent smaller volume of urine, would naturally call for a smaller water intake, but whether this can be held responsible for the

decrease of 65 to 80 per cent in the total water intake actually observed during starvation remains to be proven." My records do not support such an explanation. If water were required mainly for "excretion of nitrogenous waste products and other crystalloids by the kidney," one would not expect to find it taken so promptly after the meal.

A more plausible explanation of postprandial thirst is found in the immediate processes of digestion. Bidder and Schmidt (1852) estimated that a dog of 16 k. secreted more than 1.6 liters of gastric juice in 24 hours, i.e., a volume of fluid equal to the total blood volume of the animal. Pawlow (1889) collected 20 cc. of gastric juice in 5 minutes (200-300 cc. per hour) from a dog's gastric fistula by sham feeding. Wolfsberg (1914) fed a dog 200 grams of raw meat and obtained as a result 535 cc. of gastric juice in 3.5 hours. Dragstedt and Ellis (1930) have found that dogs with isolated stomachs secrete 1000 to 2600 cc. of gastric juice in 24 hours.

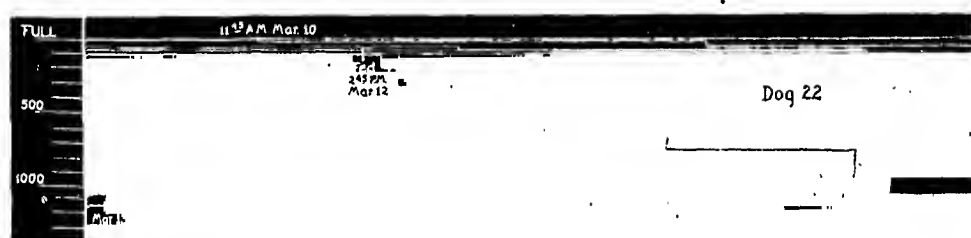


Fig. 3. Showing the effect of fasting upon the drinking of a normal dog (22). No food was given on March 10 and 11. From 11:45 a.m. March 10 until 2:45 p.m. March 12, when the animal was fed again, it drank only about 100 cc. Within five hours after the meal it drank more than 600 cc.

According to Mathews (1930) the duodenum of the dog secretes large quantities of alkaline juice; in one case 50 cc. were obtained in 2 hours from a dog weighing 5 k. In man it has been estimated that approximately 7.5 to 10.0 liters of fluid are daily secreted into the gastro-intestinal tract (Rowntree, 1922). The volume of the digestive secretions is evidently so large that their discharge may lead to a considerable decrease in the volume of the body fluids. Indeed, if in the dog reabsorption of the gastric juice is prevented by pyloric obstruction (Gamble and Ross, 1925), the body is rapidly dehydrated and the animal dies in a few days. These facts suggest that an explanation of the increased water intake after eating is to be found in the rapid secretion of digestive juices.

If the secretion of digestive juices is responsible for the postprandial thirst, it follows that the desire for water should disappear when, in the course of digestion, the secreted fluid has been largely reabsorbed, even though no water be taken after the meal. To test this proposition, dogs were fed but given no water until several hours later. The absence of

water during the meal did not prevent the animals from eating, as usual, all the food given.

Considerable variations in the results are found but in general the quantity of water taken in the 24-hour period is much reduced if the giving of water is delayed for several hours after feeding. A striking example from dog 20 is shown in figure 4. Two normal results were obtained on April 13

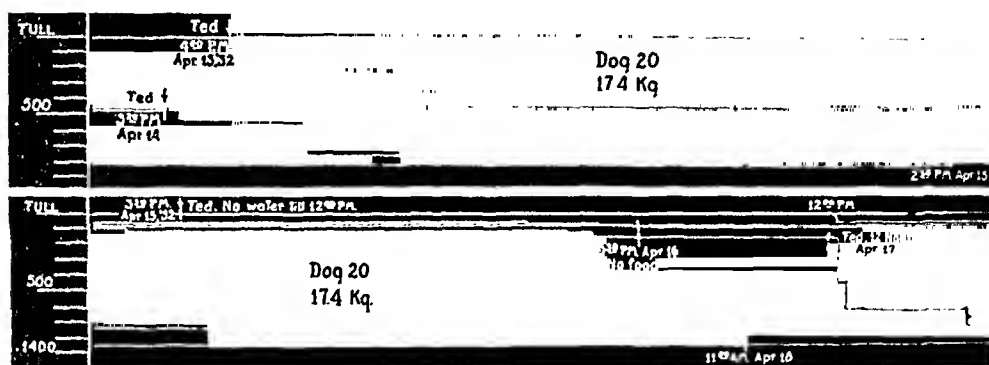


Fig. 4. Showing the drinking records obtained on dog 20 when water was not given until several hours after feeding. Normal postprandial records with water available were obtained on April 13 and 14. On April 15 food was given at 3:30 p.m. but no water until 12 midnight, i.e., 8.5 hours later. During the following 36 hours the dog drank only about 100 cc. Within 4 hours after being fed again (at noon, April 17) he drank about 700 cc.

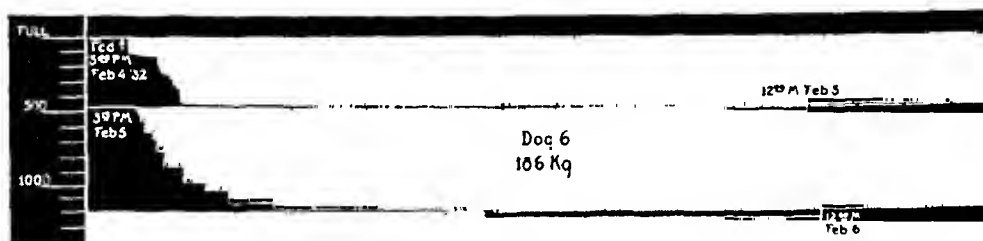


Fig. 5. Drinking records on dog 6 in which the parotid ducts were tied and the submaxillary, sublingual and infraorbital glands extirpated in October, 1931. Normal records (Feb. 4 and 5) show that most of the water is taken shortly after eating, and in spite of absence of salivary flow little is drunk between meals.

and 14. On April 15 the dog was fed at 3:30 p.m. When offered water at 12 midnight he took only a small amount, and during the next 36 hours (without food) he drank only about 100 cc. When fed at noon April 17, he drank a large amount of water (about 700 cc.) within 4 hours, as usual after eating. These and other observations (Gregersen, 1932) indicate that postprandial thirst is due to temporary dehydration resulting from the rapid accumulation of secretions in the digestive tract after food is taken.

Drinking records from dogs after exclusion of the salivary flow. In figure 5 are shown normal drinking records from dog 6 in which the parotid ducts were tied and cut between ligatures, and the submaxillary, sublingual and infraorbital glands extirpated November 24, 1931. Normally this dog drinks 500 to 700 cc. of water per day. From the records (Feb. 4 and 5) it is clear that most of this water is drunk within a few hours after food is taken. In spite of the absence of the salivary glands the dog, when not required to pant, drinks no more than normal dogs during the time between meals.

In reconsidering the purpose for which this work was begun we see that the time of day during which Gregersen and Cannon (1932) made observations on the water intake of dogs in the warm room was a period during which normal and operated dogs alike rarely drink any water. It is therefore certain that they drank because they were exposed to heat and the consequent panting made them thirsty.

Records of several different animals have purposely been included to show that the phenomena observed are not peculiarities of one particular dog.

SUMMARY AND CONCLUSIONS

1. An instrument (the potometer) is described whereby the drinking of water is recorded graphically and quantitatively (see fig. 1).

2. Graphic records of the 24-hour water intake of dogs reveal that almost all the water is taken within 2 to 5 hours after feeding, regardless of the time food is given (see figs. 2, 3 and 4).

3. Fasting usually reduces the 24-hour intake to one-fourth normal or less (see fig. 3).

4. If the giving of water is delayed for several hours after feeding, the 24-hour intake is ordinarily much less than when water is given *ad libitum* throughout the postprandial period (see fig. 4). The postprandial thirst is therefore, in part, temporary. Reasons are given for attributing it to the withdrawal of water from the body for the secretion of digestive juices.

BIBLIOGRAPHY

- BIDDER, F. AND C. SCHMIDT. 1852. *Verdauungssäfte und Stoffwechsel* (Leipzig), 36.
BINET, L. 1931. *Traité de Physiologie* (Paris), ii, 89.
DRAGSTEDT, L. R. AND J. C. ELLIS. 1930. *This Journal*, xciii, 407.
GAMBLE, J. L. AND S. G. ROSS. 1925. *Journ. Clin. Investigation*, i, 403.
GREGERSEN, M. I. 1932. *This Journal, Proceedings*, ci, 44.
GREGERSEN, M. I. AND W. B. CANNON. 1932. *This Journal*, cii, 336.
KLEITMAN, N. 1927. *Ibid.*, lxxxi, 336.
LONGET, F. A. 1868. *Traité de Physiologie* (Paris), i, 35.
MATHEWS, A. P. 1930. *Physiological chemistry*. 5th ed. (New York), 399.
PAWLOW, J. AND E. SCHUMOVA-SIMANOWSKAYA. 1889. *Centralbl. f. Physiol.*, Leipzig und Wien, iii, 113.
ROWNTREE, L. G. 1922. *Physiol. Rev.* ii, 116.
WOLFSBERG, O. 1914. *Zeitschr. f. Physiol. Chemie*, xci, 371.

ON THE MECHANISM OF PARATHYROID HORMONE ACTION

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It is now generally conceded that the increase in serum calcium and urine calcium which follows the injection of a potent parathyroid extract into a susceptible animal takes place principally at the expense of the calcium stores in the bones. The manner in which these stores are mobilized remains uncertain; three theories have been more or less explicitly put forward. It is suggested by Albright and co-workers (1930, 1932) that the parathyroid hormone lowers the renal threshold for inorganic phosphate, so that the plasma phosphate decreases and the plasma calcium is enabled to rise, the assumption being made that the plasma inorganic phosphate and calcium bear an inverse relation to one another, perhaps because the plasma behaves as if in equilibrium with solid tricalcium phosphate. The second theory has been expressed in different terms by different investigators, the central idea being that the administration of parathyroid hormone makes the plasma a better solvent for the calcium compounds of bone; for example, Greenwald (1926) suggests that there circulates in the plasma an organic substance, X , which is identical with the parathyroid hormone or is formed under its influence, and which unites with calcium ions to form an undissociated compound; so that the administration of parathyroid hormone directly or indirectly increases X , thus reducing the concentration of calcium ions in the plasma and permitting the liberation of more calcium ions from the bone; the suggestion that the plasma behaves as if in equilibrium with solid tricalcium phosphate is an integral part of the theory. The third view, which has not been thoroughly discussed from a physiological viewpoint, is that the parathyroid hormone directly stimulates cellular elements in the bone to increased osteoclastic activity. The object of this paper is to report experiments which are regarded as unfavorable to the first and second theories and hence as supporting the last.

METHODS. The experiments were carried out on unanesthetized dogs of about 20 kgm. weight, which were in most instances fasted for 18 hours before the experiment. Serum calcium was determined by the method of Clark and Collip (1925), serum inorganic phosphate by that of Fiske and Subbarow (1925), plasma pH by the colorimetric method of Cullen

(1922) with the aid of the angle centrifuge, and hemoglobin by means of the Newcomer plate.

EXPERIMENTS AND RESULTS. If the theory of Albright and his co-workers is applicable to dogs, then a rise in serum calcium produced by the injection of parathyroid extract should always be preceded or accompanied by a fall in serum inorganic phosphate. But in fact when parathyroid extract ("Parathormone" Lilly) is injected *intravenously* no such fall occurs, although the serum calcium displays the customary increase. In the two experiments illustrated in figure 1, the total serum inorganic phosphate increased slightly in one case and remained constant in the other. Since it might be urged, however, that not the total inorganic phosphate

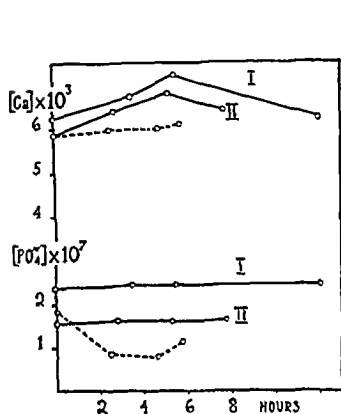


Fig. 1

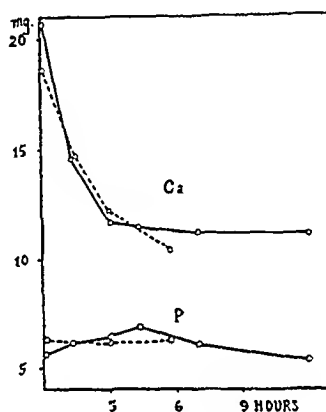


Fig. 2

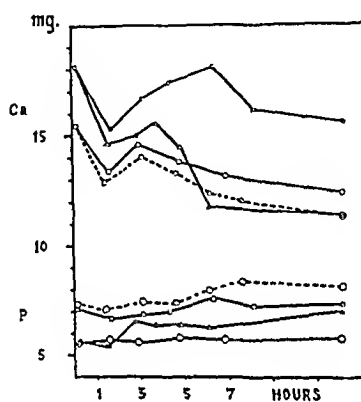


Fig. 3

Fig. 1. Serum calcium and serum $\text{PO}_4^{=}$ ion concentration after the intravenous injection of parathyroid hormone (continuous lines) and after the administration of glucose and insulin (dotted line).

Fig. 2. Serum calcium and serum inorganic phosphate, in milligram per cent, after the intravenous injection of one gram of calcium chloride.

Fig. 3. Serum calcium and serum inorganic phosphate after the simultaneous intravenous injection of one gram of calcium chloride and 100 units of parathyroid hormone.

but only the ion species $\text{PO}_4^{=}$ is of importance (in relation to the solubility product of tricalcium phosphate), the plasma pH was followed and the concentration of this species of ion calculated by means of the equation and constants of Sendroy and Hastings (1927). The continuous lines in figure 1 represent the course of two experiments of this type, in which 200 units of parathyroid hormone were administered intravenously. It is clear that the calcium, which is expressed in milli-equivalents per liter of serum, increases markedly in both cases, though, as Allardyce (1931) noted, the effect is more rapid, more transient, and less striking than after subcutaneous or intramuscular injection of the extract. On the other hand

the concentration of $\text{PO}_4^{=}$ ions, calculated from the total serum inorganic phosphate and the plasma pH and expressed in milliequivalents multiplied by 10^4 , remains strikingly constant. Parathyroid extract does not appear to produce consistent changes in plasma pH. In the same figure the dotted lines show the effects of administering 40 clinical units of insulin subcutaneously and 50 grams of glucose orally to the dog used in the first parathyroid experiment. In this case there is a very great fall in total inorganic phosphate and in $\text{PO}_4^{=}$ ions (the pH being little affected) and only an insignificant rise in calcium, not outside the limits of accuracy of the method of determination. The failure of the calcium to rise is not due to dilution of the blood, since the serum proteins and the hemoglobin decreased only trivially during the experiment. It appears then that in the dog it is possible to raise the serum calcium by injections of parathyroid extract, without lowering the serum inorganic phosphate or $\text{PO}_4^{=}$ ions, and on the other hand it is possible to reduce the total phosphate and $\text{PO}_4^{=}$ concentration greatly, by the administration of glucose and insulin, while only slightly increasing the serum calcium. It is therefore impossible to suppose that the rise in serum calcium observed in the first case is secondary to a fall in the inorganic phosphate or any part thereof, and it also appears that in experiments of short duration there is little tendency for calcium and phosphate to display an inverse relationship.

The latter point is further illustrated in one of the two experiments shown in figure 2. These experiments serve merely to show the effect of intravenous injection (at the rate of 2.5 cc. per minute) of 20 cc. of 5 per cent calcium chloride solution. The serum calcium returns to normal levels in about five hours; the total serum inorganic phosphate in one case remains stationary and in the other increases transiently. It appears to be certain that the injection of phosphate lowers serum calcium, but the reverse is evidently not invariably true.

The experiments just discussed serve as controls for those shown in figure 3, in which the animals received calcium chloride solution as before, and simultaneously 100 units of parathyroid hormone intravenously. The object of this procedure was to test the second of the theories of parathyroid hormone action; it was felt that, if the rise in serum calcium which follows parathyroid administration is due to the plasma becoming a better solvent for the bone minerals, and if therefore the serum calcium peak represents the establishment of a new equilibrium, it should be possible by supplying extra calcium at the same time as the parathyroid extract to saturate the plasma immediately, so that the bones would not be attacked, and the peak of the serum calcium time-curve should appear immediately after the injection instead of some hours later. In other words, it would be anticipated on this theory that in such an experiment the serum calcium should decrease steadily from the beginning of the

experiment, but more slowly than in the experiments in figure 2 because of the presence of excess parathyroid hormone. This expectation is not realized; on the contrary, it is perfectly clear from the shape of the curves that there is no interaction between the injected calcium and the injected parathyroid hormone; the calcium curves of figure 3 may be imitated by superimposing those of figure 1 on those of figure 2. The decrease in serum calcium in the first two hours, while the injected calcium is disappearing from the blood stream and before the action of the parathyroid extract has become manifest, is not due to dilution of the blood, since the hemoglobin content remains practically constant. The control experiments show that the second rise is not due to the removal of blood for analysis. In one of the experiments, that shown by a dotted line, the calcium chloride solution and the parathyroid extract were mixed and incubated together for twelve hours before injection; there is no evidence that they formed a compound. It will be noted again that in these experiments the rise in serum calcium is not accompanied by a fall in inorganic phosphate.

DISCUSSION. The experiments described show definitely that, in the dog, the rise in serum calcium after parathyroid injection is not secondary to any fall in phosphate; if the renal threshold for phosphate is affected, this change is not reflected in the composition of the blood. The fact that serum calcium can be slightly increased by the administration of glucose and insulin has been noted by many workers. The later experiments are unfavorable to the view that the parathyroid hormone increases the solvent power of the serum for the calcium salts of bone, though they are not irreconcilable with possible adaptations of this view. Hastings, Murray, and Sendroy (1927) showed that the power of serum to dissolve calcium salts *in vitro* was not lessened by thyroparathyroidectomy. Collip (1926) obtained double-peaked curves, similar to ours, after simultaneous injection of parathyroid hormone and calcium chloride; in his experiments the hormone was given subcutaneously, and the failure of the two curves to coalesce might have been ascribed to slow absorption; Inaba (1930) has also published similar curves, but the known unreliability of the rabbit in experiments of this type detracts from the decisiveness of his results in this particular argument. Our conclusion is, therefore, that the most acceptable theory of parathyroid hormone action is that it directly stimulates the osteoclastic process in bone. This view and its implications is more fully discussed by one of us (Thomson and Collip, 1932) in another place; it is sufficient to point out here that the histological changes seen in the bones of parathyroid-treated animals by Jaffé and Bodansky (1930), Bülbring (1931), and especially Selye (1932) suggest active destruction rather than passive solution of the bone mineral deposits.

SUMMARY

The rise in serum calcium which follows the injection of potent parathyroid extract, intravenously, in susceptible unanesthetized dogs, is not necessarily accompanied or preceded by a decrease in the total inorganic phosphate or the $\text{PO}_4^=$ ions of the serum. If such a decrease is produced by the administration of glucose and insulin, it leads only to insignificant increase in the serum calcium.

The rate of disappearance of injected calcium from the bloodstream is not apparently affected by the simultaneous injection of parathyroid extract; the subsequent action of the extract in raising the serum calcium is not apparently modified by the injected calcium chloride solution; this finding is regarded as evidence against the view that the parathyroid hormone increases the solvent power of the plasma for the calcium compounds of bone.

The view that the parathyroid hormone actively stimulates the osteoclastic process in the bone receives support from these experiments.

We desire to express our thanks to Dr. J. B. Collip for his interest in this research, and to Dr. J. S. L. Browne for valuable assistance.

BIBLIOGRAPHY

- ALBRIGHT, F. AND R. ELLSWORTH. 1930. *Journ. Clin. Invest.*, vii, 183.
ALBRIGHT, F., W. BAUER, D. CLAFLIN AND J. R. COCKRILL. 1932. *Journ. Clin. Invest.*, xi, 411.
ALLARDYCE, W. J. 1931. *This Journal*, xcvi, 417.
BÜLBRING, E. 1931. *Arch. exp. Path. u. Pharm.*, clxii, 209.
CLARK, E. P. AND J. B. COLLIP. 1925. *Journ. Biol. Chem.*, lxxiii, 461.
COLLIP, J. B. 1926. *Medicine*, v, 1.
CULLEN, G. E. 1922. *Journ. Biol. Chem.*, lii, 501.
FISKE, C. H. AND Y. SUBBAROW. 1925. *Journ. Biol. Chem.*, lxxvi, 375.
GREENWALD, I. 1926. *Journ. Biol. Chem.*, lxxvii, 1.
HASTINGS, A. B., C. D. MURRAY AND J. SENDROY. 1927. *Journ. Biol. Chem.*, lxxi, 723.
INABA, M. 1930. *Journ. Biochem., Japan*, xii, 35.
JAFFÉ, H. L. AND A. BODANSKY. 1930. *Journ. Exp. Med.*, lii, 669.
SELYE, H. 1932. *Endocrinol.*, in press.
SENDROY, J. AND A. B. HASTINGS. 1927. *Journ. Biol. Chem.*, lxxi, 783.
THOMSON, D. L. AND J. B. COLLIP. 1932. *Physiol. Rev.*, xii, 309.

THE EFFECTS OF THE GONADAL-STIMULATING HORMONE OF THE ANTERIOR PITUITARY ON THE VOLUNTARY ACTIVITY, THE AGE OF MATURITY AND THE SIZE OF THE LITTER IN IMMATURE FEMALE ALBINO RATS

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Earlier investigators have proven that in normal female rats there is an increased voluntary activity coinciding with the heat or oestrous period, which occurs on the average every fourth or fifth day (Long and Evans, 1922; Wang, 1923; Slonaker, 1925; Durant, 1924, 1925; Hitchcock, 1925, and Bugbee and Simond, 1926). It seemed that if it was the gonadal-stimulating hormone of the pituitary that produced these rhythmic heat periods in the adult female animal, then this same hormone, when injected into immature female rats, might produce an increased voluntary activity, or cause rhythmic variations of activity similar to that found in the normal matured animal. It has been experimentally proven (Smith and Engle, 1927; Zondek and Aschheim, 1927) that sexual maturity can be induced in immature animals (rats and mice) by means of transplanting anterior lobe pituitary gland either subcutaneously or intramuscularly. The number of transplants required to produce the effect depends upon the age of the rat and varies in individuals.

These investigations were carried out to determine the effects of the pituitary hormone on voluntary activity, age of puberty, and the size of the litters.

PROCEDURE. Immature female albino rats ranging from eighteen to twenty-two days of age were used as subjects for this experiment.

The technique was that employed by Smith (1927). Immature and mature rats of both sexes were used as donors of the pituitary tissue. While it has been shown by Engle (1929), and Evans and Simpson (1929, 1929a), that the potency of pituitary substances varies with age, sex and physiological conditions, no account of this fact was taken in this work. In all cases sufficient material was injected to produce the desired effects regardless of the potency of the transplants.

The recipients were divided into four different classes and each of these, except group I, were divided into subdivision A and subdivision B. All the animals in subdivision A were the progeny of mothers that matured

normally; those in subdivision B were the progeny of precocious sexually matured mothers. The first class comprised thirty-two animals that were injected with two glands per day for two successive days. In the second class (subdivision A—sixty animals, subdivision B—sixty animals), two glands were injected each day for two successive days followed by a second injection of three glands five days after the last injection and this followed by a third injection of three glands four days after the second injection. In the third class (subdivision A—seventy animals, subdivision B—eighty animals), each animal received daily, until the vaginal canal opened, 1 cc. of an extract D "which contained a considerable amount of the gland material in the form of an emulsion." Animals in the fourth class (subdivision A—one hundred and ten animals, subdivision B—seventy animals) were likewise injected subcutaneously each day, until the vaginal canal opened, with 2 cc. of an extract A "that had been filtered through a porcelain filter." These two extracts were products of Parke, Davis & Company.

In all the series littermate sisters were used as controls. In addition to fifteen untreated controls, 180 others were treated with an amount of normal physiological saline solution corresponding to either the amount of gland transplant or extract injected into the experimental animal. There might be some criticism of controls injected with normal physiological saline solution but the work from this laboratory has shown that the injection of corpus luteum material (Durrant, 1926), theelin, amniotin, whole ovary, and ovarian extract (Durrant et al., unpublished) has failed to produce the characteristic effects shown when pituitary substances were injected.

Immediately after the first injection the rats were put into separate activity cages, similar to those described by Durrant (1924, 1925) and Hitchcock (1925). The cyclometer of each cage was read daily at approximately the same hour.

The animals were weighed weekly throughout the period of experimentation, the first weight being recorded previous to the first injection. There was no appreciable difference in weight between the experimental animals and the controls.

The criteria used in judging sexual maturity were: first, the canalization of the vagina; and second, the bearing of young. Following the injections, the animals were observed hourly to note the time required for the opening of the vaginal canal. After the opening of the canal, vaginal smears were taken daily (Stockard and Papanicolaou, 1927). In this way the presence or absence of a normal oestral cycle was noted.

In order to investigate the youngest possible age for bearing young, a group of immature female rats were brought to sexual maturity at the age of twenty-three days with gland transplants, in the manner already

described. The smallest males, that were thought to be sexually matured, were introduced into the cages containing the young females. Care was taken to prevent the males from killing or injuring the females, though this happened in some cases. Artificial introduction of sperms was successful with three animals. Two accepted coitus.

In determining the effect of pituitary injections on the size of litters precocious sexually matured females were bred at ages ranging from fifty to sixty days. Pregnant animals were inspected daily and the sexes of the offspring were recorded at birth (Jackson, 1912). The size of litters and the sex ratios obtained were compared with similar data from normal mothers. Data for stillborn young have been included.

TABLE 1

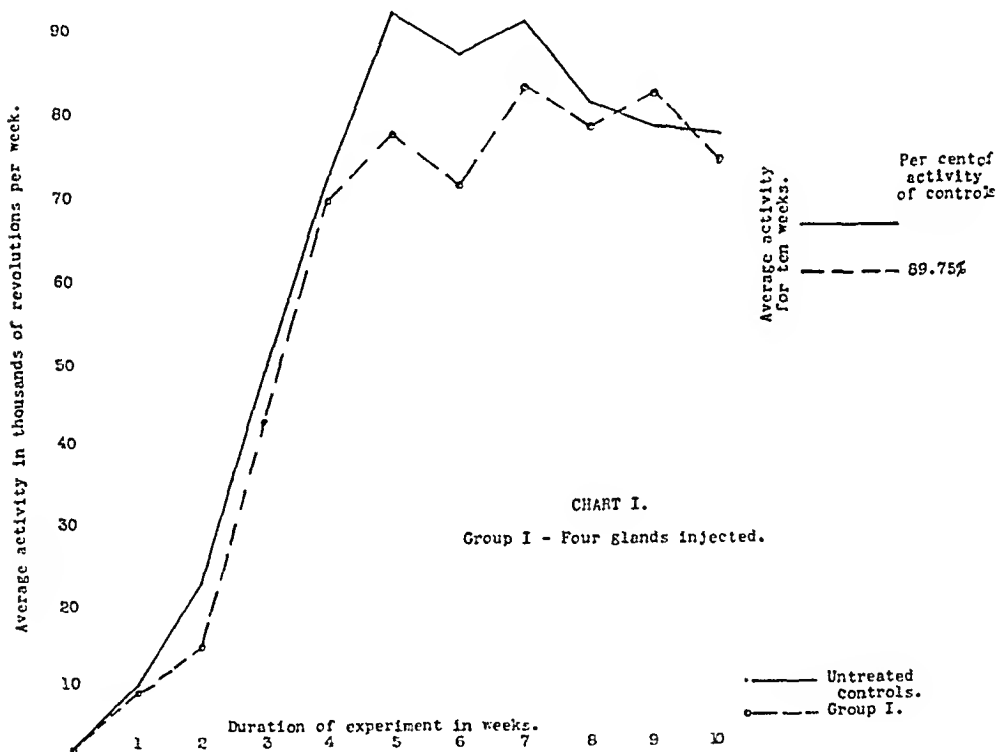
Table showing the average age at which the vaginal canal opened with per cent variation from controls

	TOTAL NUMBER OF ANIMALS	AVERAGE AGE AT OPENING OF VAGINAL CANAL	VARIATION IN EACH SUBDI- VISION	AVERAGE AGE AT OPENING OF VAGINAL CANAL IN EACH GROUP	VARIATION IN EACH GROUP
			<i>per cent</i>		<i>per cent</i>
Control { A.	249	34.47		32.86	
{ B.	81	28.80			
Group 2 { A.	220	23.40	67.88	22.79	69.35
{ B.	120	21.66	75.21		
Group 3 { A.	70	21.51	62.58	21.80	66.34
{ B.	100	21.00	72.92		
Group 4 { A.	150	25.31	73.43	24.04	73.16
{ B.	70	21.43	74.41		

RESULTS AND DISCUSSION. *Activity.* Since the purpose of this experiment was to determine the effect of the injected material on the voluntary activity of immature rats, the most significant activity would be that recorded before the normal age of puberty. The average age of maturity among the controls was 34.47 days (see table 1). The significant voluntary activity would, therefore, be that of the first two weeks of experimentation. Judged on the activity of these first two weeks the curves obtained when the daily activity of individual experimental animals was plotted failed to show typical oestrous cycles. A few peaks that might be interpreted as heat periods occurred, but since such peaks were more numerous among the controls than among the experimental animals, it must be concluded that the various injected substances failed to produce the cyclic variations in activity characteristic of the matured female.

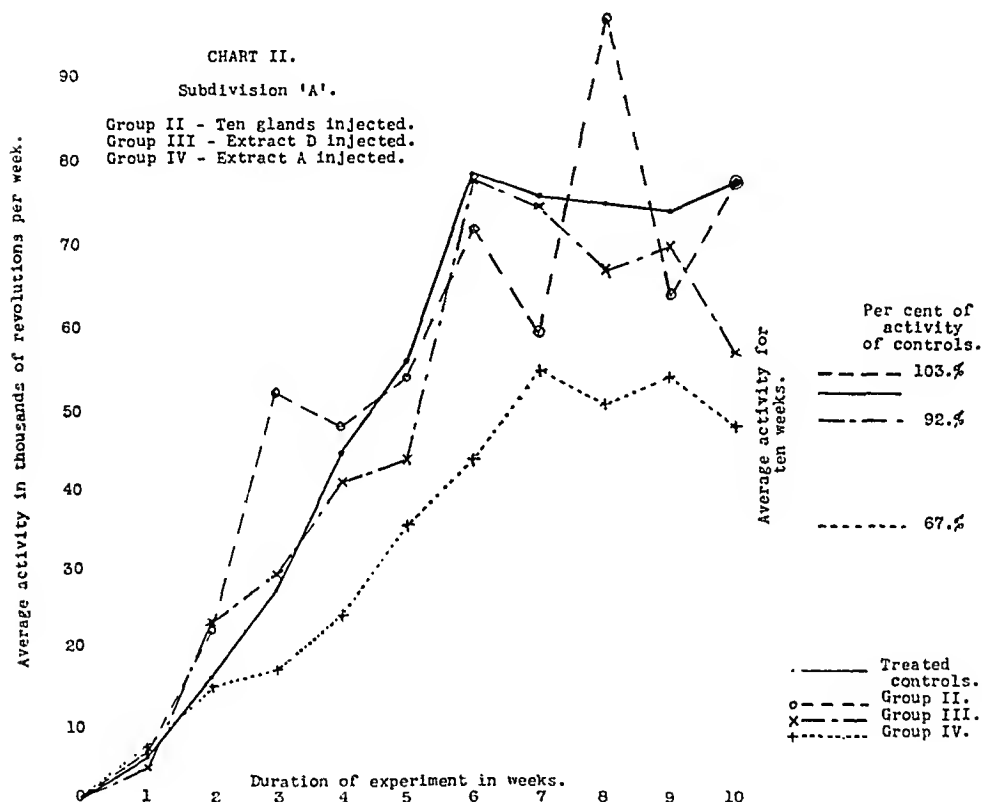
In judging the effect of the various substances injected on the total voluntary activity records were kept for a period of ten weeks, as it was felt that effects produced before or at puberty might very well persist for some time. The data obtained are shown graphically in charts I, II and III.

It is apparent from chart I that the activity of the animals receiving gland transplants was slightly less than that of the controls. The average for the entire period of the ten weeks is a shade less than ninety per cent of the activity of the control group. This decrease is due largely to a falling off of the activity of the experimental animals in the fifth, sixth and seventh weeks of the experiment.



It would seem from an inspection of chart II that the only outstanding effect in voluntary activity produced was a decrease in the activity of the animals receiving extract A. These animals showed an activity only 67 per cent of that of the controls. While there are certain variations in the activity of the animals in groups II and III, it is doubtful if these variations are significant. An inspection of chart III makes the significance of the variations occurring in the activity of groups II and III seem even more doubtful. It must be remembered that these animals were the offspring of female rats that had been prematurely brought to sexual maturity by

gland transplants. On this chart the animals receiving extract D showed an activity greater than the controls, while those receiving gland transplants were slightly less active than the control group. This is just the reverse of the results obtained with the animals whose activity had been plotted in chart II. Therefore, the only conclusion which can be safely drawn is that the injection of pituitary glands or of extract D produces no significant change in the total voluntary activity. However, since the animals injected with extract A showed an activity well below that of the controls

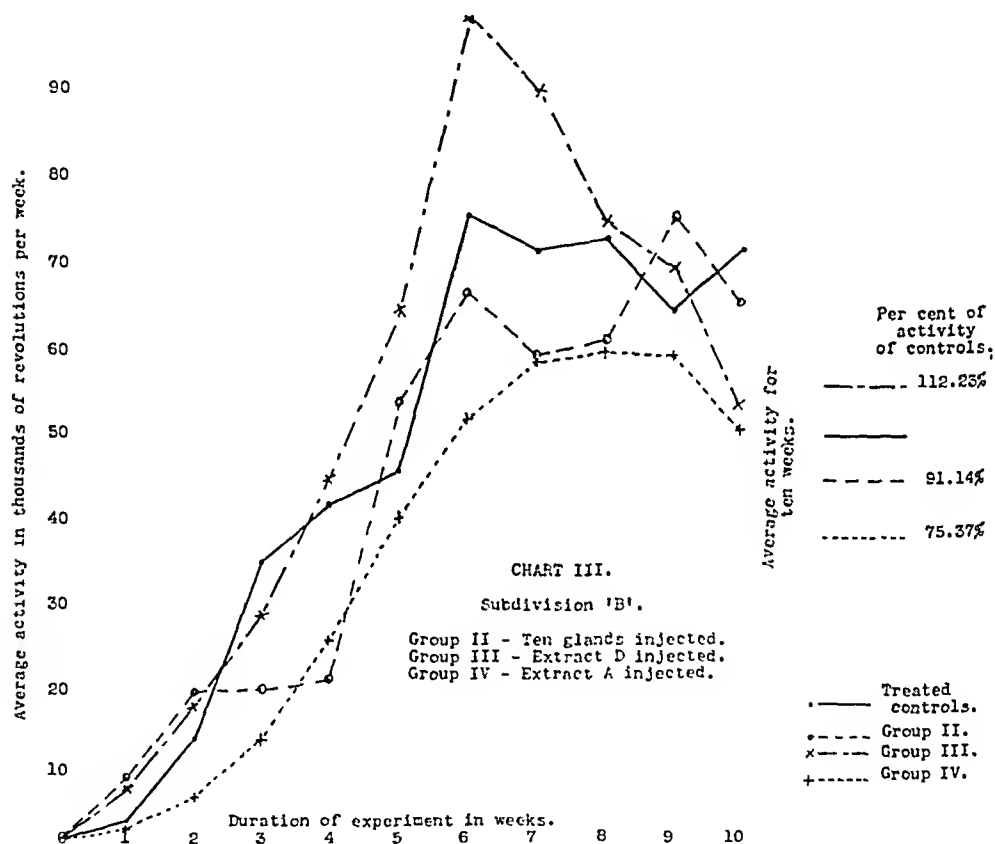


in both groups, it appears justifiable to conclude that this extract depressed the voluntary activity.

Average age at which vaginal canal opened. Table 1 shows the ages at which the vaginal canal opened in the experimental animals and their controls. As in the other parts of the experiment, the animals were divided into two groups. Those animals whose mothers became matured normally constituted subdivision A, while those whose mothers were brought to sexual maturity by the use of pituitary substances constitute subdivision B. In more than 700 animals there was no case in which the treatments used in these experiments failed to induce sexual maturity, as judged by

the canalization of the vagina. Usually two to three days were required. However, with animals twenty days old at the beginning of the treatment, sexual maturity has been induced within seventeen hours by the injection of two gland transplants. No animals less than eighteen days old were used.

It will be noticed that those animals in subdivision B required a shorter treatment than did those in subdivision A. In the case of gland transplants there was an average difference of 1.74 days; with extract A, a



difference of 3.88 days; and with extract D, a difference of 0.54 day. Even the controls showed a decided difference of 5.69 days.

A matured genital system as judged by the canalization of the vagina was secured in thirty animals at nineteen days of age by the injection of 1 cc. of extract D. The animals were treated on their eighteenth day of age. This is considerably younger than any reported case of maturity in normal animals. According to Long and Evans (1922) a normal female rat matures on the average at seventy-two days of age, the range being from thirty-four to one hundred and nine days. According to Donaldson

(1924) the age of puberty is two months. In this experiment the average age at which normal controls matured was 34.47 days.

In this series the opening of the vaginal canal occurred in the experimental animals at ages ranging from nineteen to twenty-eight days. Smith (1927, 1930) reported the youngest age at which he was able to secure a fully matured genital system in the rat to be twenty-two days (the weaning date), treatment having been instituted at the age of fourteen days.

The results obtained from attempts to breed precocious sexually matured animals offer additional evidence that these animals were truly brought to sexual maturity. Five precocious sexually matured females were successfully bred. In three of these the sperm was artificially introduced, while two accepted coitus. These five females bore young at ages ranging from forty-eight to fifty days. Their weights were between 86 and 102 grams. According to Donaldson (1924) the period of gestation is usually from twenty-one to twenty-two days. If this be taken as a standard, the age of impregnation in these females was twenty-six to twenty-eight days. As far as could be found this is the earliest age at which female rats have borne young.

Vaginal smears. Vaginal smears were taken daily on all animals for fifty to seventy consecutive days. In no case was there evidence of a typical cyclic change in the experimental animals. The most frequent smears showed a typical dioestrous cell content. However, frequently a mixed type of cornified and nucleated epithelial cells and leucocytes occurred, in which the proportion of these cells varied from day to day and gave some indications of cyclic changes.

The fact that this mixed type of smear was so frequently found might indicate that instead of the usual period of about five days (Long and Evans, 1922) the oestrous cycle was shortened to a period of one to two days, thus reducing the various stages from days into hours. There is without doubt a decidedly rapid rate of growth of the genital organs in these animals and the accelerated oestral cycle could be easily explained on this basis.

This accelerated oestral cycle may explain the failure to obtain activity records indicative of true heat periods. Another possibility is that the luteinization of the ovary produced by the gonad-stimulating hormone of the pituitary interfered with the normal oestral cycle.

Size of litter. The size of the litters from mothers made sexually mature at an early age was larger in every experimental group than that of the controls. This difference was more pronounced in subdivision B than A (see table 2). Since the animals in subdivision B were the offspring of mothers who had been brought to sexual maturity by gland transplants, the young of these females were the offspring of two generations of treated females.

In subdivision A the average number of young in a litter for the controls was 5.96, based upon forty-five litters. The average number of young born from mothers who had been treated with extract D was 7.34 (21 litters). The average number of young based on twelve litters born from mothers treated with extract A was 10.17, and that from mothers treated with gland transplants was 10.13, taken from twenty-three litters. These differences are significant.

In subdivision B even a greater difference existed. The progeny that received extract D bore on the average 9.10 young per litter (21 litters), those treated with extract A, 9.55, (11 litters), and those treated with gland transplants, 11.50, (20 litters). In twenty-nine control litters the average number of young was 7.97. Combining all the litters in subdivision A we have fifty-six litters with a total of 507 young which is an average of 9.05 young per litter.

TABLE 2
Tabulation of the size of the litters and the sex ratio

	NUMBER OF LITTERS	NUMBER OF MALES	NUMBER OF FEMALES	TOTAL NUMBER OF YOUNG	AVERAGE NUMBER OF YOUNG PER LITTER	NUMBER OF MALES PER 100 FEMALES
<i>Normal mothers:</i>						
Controls.....	45	164	104	268	5.96	157.68
Experimentals.....	56	183	324	507	9.05	56.48
<i>Treated mothers:</i>						
Controls.....	29	123	108	231	7.97	113.89
Experimentals.....	52	170	356	526	10.11	47.75

Treating the animals in subdivision B in a similar way we have fifty-two litters with a total of 526 young, which is an average of 10.11 animals per litter. It is apparent, therefore, that the effect is more pronounced in the second generation than in the first.

It appears, therefore, that animals treated with pituitary substance are able to transmit some factor that enables their progeny to attain sexual maturity earlier and to show a higher degree of fertility. The transmitted factor is perhaps a greater susceptibility to the hormone of the anterior lobe of the pituitary.

The data contained in table 2 also seem to indicate that injections of pituitary substances decreases the sex ratio. In the three groups of subdivision A there were a total of 183 males to 324 females born in fifty-six litters. This is a ratio of 56.4 males to 100 females. In subdivision B where the young are offspring of two generations of pituitary treatment there were 170 males to 356 females in fifty-two litters. This is a sex

ratio of 47.8 males to 100 females. The sex ratio among the controls of subdivision A was 157.7 males to 100 females, a considerably higher ratio than previously reported. However, in the controls of subdivision B the sex ratio was 113.9 males to 100 females. It must be remembered that while none of the mothers in the control group received treatment, the grandmothers of the control animals in subdivision B had been brought to precocious sexual maturity by gland transplants. A study of these figures seems to indicate that continued treatment in succeeding generations with pituitary substance tends to decrease the relative number of males born. These figures are based on more than 1500 young. While it is perhaps unwise to draw definite conclusions from this number it is felt that the data involved are sufficient to justify the conclusion that treatment with pituitary substance tends to decrease the sex ratio.

SUMMARY

Anterior-pituitary tissue and extracts of the gland were injected into sexually immature female albino rats and the following effects noted:

1. The total voluntary activity was not significantly changed except in the group receiving extract A where there was a marked decrease in the total activity. The voluntary activity of the experimental animals showed no cyclic variations such as those reported by earlier workers on normal sexually matured females.

2. Vaginal smears also failed to show the typical oestral cycle. The failure to obtain evidence of a typical oestral cycle may be due to luteinization of the ovary or to a marked shortening of the oestral cycle.

3. A total of 730 immature female rats received injections of pituitary substance and in every case precocious sexual maturity was produced as evidenced by 1, the canalization of the vagina. This occurred in from seventeen hours to three days following treatment. 2, the experimental animals conceived and bore young. In one case young were produced when the mother was only forty-eight days old.

4. Litters of animals that had received experimental treatment were much larger than the litters of the controls.

5. The sex ratio in the offspring of the experimental animals was much lower than that in the controls, i.e., there was a larger per cent of females in the young of the treated mothers than in the controls.

6. All of the effects noted were more pronounced in the second generation of experimental animals. This may mean that at least a part of the effect is transmitted to the young.

The author wishes to express her appreciation to Dr. L. B. Nice under whose direction the investigation was carried out, and to Parke, Davis & Company for supplying the extracts used.

BIBLIOGRAPHY

- BUGBEE, E. P. AND A. E. SIMOND. 1926. *Endocrinol.*, x, 360.
- DONALDSON, H. H. 1924. *The rat*. Mem. Wistar Inst., no. 6. Philadelphia.
- DURRANT, E. P. 1924. *This Journal*, lxx, 344.
1925. *Endocrinol.*, ix, 221.
1926. *Endocrinol.*, x, 286.
- DURRANT, E. P. ET. AL. Unpublished.
- ENGLE, E. T. 1929. *This Journal*, lxxxviii, 101.
- EVANS, H. M. AND M. E. SIMPSON. 1929. *This Journal*, lxxxix, 371.
1929. *This Journal*, lxxxix, 375.
- HITCHCOCK, F. A. 1925. *This Journal*, lxxv, 205.
- JACKSON, C. M. 1912. *Biol. Bull.*, xxiii.
- LONG, J. A. AND H. M. EVANS. 1922. *Mem. Univ. Calif.*, vi.
- SLONAKER, J. R. 1925. *This Journal*, lxxi, 362.
- 1925a. *This Journal*, lxxiii, 485.
- SMITH, P. E. 1927. *This Journal*, lxxx, 114; lxxxi, 27.
1930. *Amer. Journ. Anat.*, xlv.
- SMITH, P. E. AND E. T. ENGLE. 1927. *Amer. Journ. Anat.*, xl, no. 2.
- STOCKARD, E. AND G. N. PAPANICOLAOU. 1927. *Amer. Journ. Anat.*, xxii, 225.
- WANG, G. H. 1923. *Comp. Psychol. Monographs.*, ii, no. 6.
- ZONDEK, B. AND S. ASCHHEIM. 1927. *Klin. Wochenschr.*, vi, 248.
1927. *Arch. F. Gynäk.*, cxxx, 1.
1927. *Klin. Wochenschr.*, vi, 1321.

STUDIES ON SUBCUTANEOUS ABSORPTION

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In spite of the universal practice of subcutaneous injection in both clinical and experimental work, review of the literature reveals no reference to a study of the rate and mechanism of absorption of subcutaneously injected substances. In an effort to throw light on these questions, determinations have been made as to the amounts of dihydroxyacetone, glucose, fructose, and galactose absorbed in a given time after subcutaneous injection.

METHOD. The method of procedure of these experiments was as follows: known amounts of the four substances, dihydroxyacetone, glucose, fructose, and galactose, were injected subcutaneously in a series of rats. The injections were made into the lumbar region using the Record type of syringe since it could be adjusted so that exactly the same volume, approximately 1 cc., could be injected each time. The same amount of solution was expressed into a flask and analyzed by the method of determination used throughout these experiments. In this way the exact amount of material injected into the rats was determined. The animals were sacrificed at varying periods after injection and the skin was dissected away to recover the unabsorbed material, which was then quantitatively determined. The amount absorbed was then calculated by subtracting the unabsorbed quantity from the total amount injected.

These experiments consist of two series. In the first series, 50 per cent solutions of the four substances were used. The animals were killed immediately and after fifteen, thirty, and forty-five minute periods of absorption. The determinations were performed by the Shaffer-Hartmann method.

The results of the first series of experiments were such that it was deemed essential to perform a second series using more dilute solutions. Both 2.5 per cent and 5.0 per cent solutions of glucose were used. The recovered samples in the second series were analyzed by the method of Hagedorn and Jensen (1923). Control injections of physiological saline were made in

¹ The data here presented are taken from the theses submitted by G. S. Goldman and M. Y. Krosnick in partial fulfillment of the requirements for the degree of Doctor of Medicine, Yale School of Medicine, in 1929 and 1930 respectively.

order to ascertain whether the results were being affected by reducing substances from the tissue fluids, and if so, to what extent. These values were used to correct the apparent values for glucose.

RESULTS. Ninety-two experiments were performed with the concentrated solutions: 40 with glucose, 19 with dihydroxyacetone; 17 with fructose; and 16 with galactose. The results are summarized in table 1. Graphs were constructed for each of the substances plotting the percent absorbed against the period of absorption in minutes, using the data of table 1. In each case the best curve drawn through the four points was a straight line. The final results considered in the discussion are those obtained from the curve rather than the amounts obtained experimentally at the end of forty-five minutes.

The results of the second series of experiments, sixteen with 2.5 per cent glucose and thirty-six with 5.0 per cent solution, are shown in table 2. These values have been corrected for the amounts of reducing substances found after saline injection. The curves to be obtained by plotting these

TABLE 1
Per cent absorbed from 50 per cent solutions in varying periods of time

	15 MINUTES	30 MINUTES	45 MINUTES ACTUAL DETER- MINATION	45 MINUTES ESTIMATED FROM CURVE
Dihydroxyacetone.....	21.6 \pm 2.2	37.0 \pm 2.2	57.6 \pm 1.9	59.3
Glucose.....	13.7 \pm 2.0	37.5 \pm 1.6	45.0 \pm 1.4	48.0
Galactose.....	13.4 \pm 2.8	26.7 \pm 2.7	37.5 \pm 2.5	39.2
Fructose.....	10.9 \pm 1.8	23.7 \pm 2.1	43.0 \pm 2.2	37.0

results are hyperbolic in contrast to the straight lines for concentrated solutions.

DISCUSSION. The absorption of the 50 per cent solutions of triose and the three sugars for a period of 45 minutes is as follows: dihydroxyacetone, 59.3 per cent; glucose, 48.0 per cent; galactose, 39.2 per cent; and fructose, 37.0 per cent. The significance of the differences between these values depends upon the error involved in the injection and recovery of the sugars since the error of the chemical analysis is negligible—less than 0.5 per cent. By referring to table 1, it is found that the average deviations from the mean for each group of experiments range between 1.4 and 2.8. It is obvious, therefore, that the difference between dihydroxyacetone and glucose is significant, as is that between glucose and the other sugars. The difference between galactose and fructose, however, is well within the error of the method.

The first fact indicated by the data of the experiments with 50 per cent solutions is that these substances injected subcutaneously are absorbed

at different rates. Nagano (1902) found that the rate of absorption from the intestine of stereo-isomeric sugars is different, but Hewitt (1924) observed this difference disappeared when the epithelial lining was destroyed with hot saline or fluoride. In this case where no epithelial lining is involved the difference may be ascribed to variations in molecular configurations and activities. The dihydroxyacetone molecule, in particular, is smaller than the hexose molecule. The second point is that the rate of absorption for a given sugar remained constant throughout the duration of the experiment; i.e., the curve of the rate of absorption is a straight line. This phenomenon is regarded by Cori (1925) as characteristic of the absorption of hexoses and pentoses as it occurs in the intestine, lined by a highly specialized epithelial membrane, though Pierce, Osgood, and Polansky (1929) could not confirm such a finding for glucose. Cori and Cori (1929) did not find a constant rate of intestinal absorption of the triose lactic acid. However, no specialized absorptive membrane in the subcutaneous tissues has ever been described; and therefore it seems improbable, in spite of the present evidence, that absorption would take

TABLE 2
Per cent of injected substance absorbed in varying periods of time

	15 MINUTES	30 MINUTES	60 MINUTES
2.5 per cent glucose.....	40.5 \pm 1.4	56.9 \pm 1.8	62.0 \pm 2.6
5 per cent glucose.....	23.6 \pm 0.7	41.1 \pm 2.6	53.9 \pm 4.6

place at a constant rate. Instead, the rate of absorption should diminish as the amount of unabsorbed material decreases as in absorption from the peritoneal cavity. It seemed probable that this unexpected result was due to the high concentration of the solutions injected. When the solution is very concentrated as soon as one molecule has been absorbed from the surface of the tissue another takes its place. Thus there may be so many molecules present that there would be no diminution in the rate of absorption for the duration of the experiment. That this is the case is indicated in a few experiments performed with 25 per cent glucose solutions in which the rate of absorption was found to be essentially the same as that for 50 per cent solutions. In view of these results it is probably a fact of lesser importance that the 50 per cent solution of hydroxyacetone used in the experiment contained twice as many molecules as the 50 per cent hexose solutions.

However, when more dilute solutions of glucose were used, namely, 2.5 per cent and 5 per cent, the expected diminishing rate of absorption was found as illustrated by hyperbolic curves obtained by plotting the results. This type of absorption is similar to that observed in the intestine for

lactic acid (Cori and Cori, 1929) and for glucose (Pierce, Osgood, and Polansky, 1929). A diminishing rate of absorption has also been observed in the peritoneal cavity (Cori and Goltz, 1925).

When the animals were killed immediately after the injection of concentrated solutions the total amount of the substance injected was not recovered. The amount not recovered was constant for each substance and so this value could be subtracted from the quantity unrecovered after a definite period of absorption to obtain the amount actually absorbed during that period. The amounts that could not be recovered when the animal was killed immediately are as follows: dihydroxyacetone, 27.0 per cent; glucose, 15.7 per cent; fructose, 11.8 per cent; galactose, 10.7 per cent.

An additional correction factor was required in the experiments with the 5 per cent and 2.5 per cent glucose solutions for quantities of reducing substances, which are significant because of the dilute solutions used, diffuse into the injected solution. It was, therefore, thought advisable to subtract the amount of reducing substances found in control saline injections from the results obtained when glucose was injected. The objection may be made that the infiltration of reducing substances into an injected saline solution is not necessarily indicative of an equal inflow when glucose is injected. However, even without the use of the corrective factor the absorption rate curves for these dilute solutions are hyperbolic, indicating a diminishing rate of absorption, but the use of the corrective factor brings out more pronouncedly the character of the curves.

SUMMARY AND CONCLUSIONS

1. In order to determine the rate of absorption of dihydroxyacetone, glucose, fructose, and galactose, ninety-two experiments were performed in which 50 per cent solutions of these substances were injected subcutaneously. In addition a 2.5 per cent solution of glucose was used in sixteen experiments and a 5 per cent solution in thirty-six.

2. At the end of 45 minutes, the per cent absorbed for each of the 50 per cent solutions was as follows: dihydroxyacetone, 59.3 per cent; glucose, 48.0 per cent; galactose, 39.2 per cent; and fructose, 37.0 per cent. After one hour 62.0 per cent of the 2.5 per cent solution of glucose had been absorbed and 53.9 per cent of the 5 per cent solution.

3. The results indicate first, different rates of absorption for these substances; second, an apparently constant rate of absorption for each substance from a concentrated solution (50 per cent), and third, a diminishing rate of absorption from a dilute solution (5 per cent and 2.5 per cent).

We are glad to thank Miss Marjorie H. Hurlburt for aid in the preparation of this paper.

BIBLIOGRAPHY

- CORI, C. F. AND H. L. GOLTZ. 1925. *Proc. Soc. Exp. Biol. and Med.*, xxii, 122.
- HAGEDORN, H. C. AND B. N. JENSEN. 1923. *Biochem. Zeitschr.*, cxxxv, 46.
- HEWITT, J. A. 1924. *Biochem. Journ.*, xviii, 161.
- NAGANO, J. 1902. *Pflüger's Arch.*, xc, 389.
- SHAFFER, P. A. AND A. F. HARTMANN. 1921. *Biol. Chem.*, xlv, 365.
- CORI, C. F. 1925. *Biol. Chem.*, lv, 691.
- CORI, C. F. AND G. T. CORI. 1929. *Biol. Chem.*, lxxxi, 389.
- PIERCE, H. B., H. S. OSGOOD AND J. B. POLANSKY. 1929. *Journ. Nutr.*, i, 247.

A STUDY OF CUSHNY'S THEORY OF SULPHATE DIURESIS

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The experiments reported in this paper constitute an attempt to apply Cushny's theory of urine secretion to a specific case. Experiments of this type do not, of course, afford direct evidence of the validity of a theory until they reveal the limits of its applicability, and thus compel modification or rejection. The work was undertaken therefore to see how far the theory could be applied. Cushny's theory was accordingly assumed to be correct and used for making calculations from the experimental data.

As this theory is so well known, it is unnecessary to do more than point out the particular assumptions of the theory made use of in the calculations. Following Rehberg (1926) it is assumed that the creatinine of the urine is derived from the plasma exclusively by filtration and that none of it is reabsorbed. This assumption is used to calculate the amount of filtrate and reabsorbed water. In addition simultaneous samples of blood from the aorta and from the renal vein were taken which permit not only the calculation of the per cent of blood filtered off in the glomeruli, but also estimations of the rate of blood flow through the kidney. We thus have figures for the blood flow, the percentage filtration to the glomeruli as well as the total filtrate, and the amount reabsorbed in the tubules.

Cushny's explanation of sulphate diuresis (p. 149) is that in the first stage the diuresis arises from colloid-dilution and increased flow through the renal capillaries, while as the diuresis passes off the influence of sulphate in the tubules in preventing reabsorption becomes more prominent. It is obvious that now we are in a position to obtain numerical data for these three factors.

METHODS. Experiments were performed on dogs under amytal anesthesia. Although this drug has some action on the kidney (1930) the effects during sulphate diuresis are comparatively small as shown by the latency of water excretion. The amytal was administered intravenously.¹ As soon as complete anesthesia was obtained, the abdomen was opened and the kidneys exposed. In nearly every case one kidney (usually the right)

¹ In some cases sodium amytal was used, for which we are indebted to the Eli Lilly Company.

was isolated by tying off the vessels and the ureters. A cannula was then inserted into the bladder. When both kidneys were allowed to act, ureteral catheters were used. In some cases the carotid blood pressure was recorded. A cannula was then inserted into the femoral vein and through it the animal was given about 200 cc. of 5 per cent sodium sulphate solution and 1.25 gram creatinine dissolved in 25 cc. of water. (The reason for giving the creatinine is the one mentioned by Rehberg; viz., to decrease the error in its estimation in the blood due to the presence of other substances giving the same color.) Collection of urine was begun immediately after the completion of the injection. Samples of blood were taken half an hour later from the abdominal aorta and the renal vein with a needle and syringe, and this was repeated at hourly intervals. The urine was also collected in hourly periods so that the blood samples represent the values in the middle of the periods of urine collection. In half the experiments creatinine estimations were done on whole blood and in the other half plasma was used.

The distribution of creatinine between corpuscles and plasma after creatinine injection has been investigated by Berglund (1922) and by Underhill (1923) who find that the values for plasma and whole blood are usually very nearly the same. We have confirmed this, but we find in agreement with these authors that differences do occur in either direction. Consequently when the figures for whole blood are used, the values for filtrate, etc., may be somewhat in error, but the values for percentage filtered and blood flow will be more exact. When the plasma figures are used, the opposite errors may occur, but it does not seem probable that the magnitude of the error will be such as to make any significant difference in interpretation.

In the first place the percentage of blood filtered may be considered. The exact derivation of the figures for percentage filtration should be borne in mind. They represent the difference in creatinine content of arterial and renal vein blood reckoned as a percentage of the former. The creatinine which is filtered is entirely in the plasma. It is obvious that the process of filtration will not alter the concentration in either plasma or corpuscles, but as the blood travels past the tubules it will be diluted with a considerable volume (perhaps 15 per cent of the total blood volume) of a fluid which is presumably creatinine free. This alters the concentration of the plasma which in turn will produce changes in the corpuscles as a new equilibrium is set up. If this equilibrium is attained by the time the separation of plasma is carried out, then the figures for percentage filtration will be identical whether calculated from whole blood or plasma. The experiments reported at the present time do not settle this point completely, but it will be seen that there is no significant difference between the two sets of values.

It is of interest to compare estimates of per cent filtration derived in this way with those of Mayrs (1922) who derived his figures from the plasma and urine sulphate, the urine volume and blood flow. He finds that the percentage usually lies between 20 and 25 per cent but may vary from 6 to 40 per cent. Gibbs (1928) found that the kidney could remove 30 per cent of the uric acid passing through it. Our figures are somewhat lower. With regard to whole blood the highest value recorded is 20 per cent (expt. 1, table 1). The usual range is between 8 and 15 per cent. In two out of the four experiments the percentage decreases as the diuresis passes off, and in the other two it does not. In experiment 1, for instance, it rises from 12

TABLE 1
Analyses of whole blood

EXPERIMENT NUMBER	WEIGHT OF KIDNEY	SAMPLE NUMBER	URINE VOLUME	URINARY CREATININE	BLOOD CREATININE		FIL- TRATE	REAB- SORBED	PER CENT FIL- TERED	PER CENT NOT REAB- SORBED	BLOOD FLOW
					Arte- rial	Renal volume					
	<i>grams</i>		<i>cc./hr.</i>	<i>mgm./ hr.</i>	<i>mgm./ 100 cc.</i>	<i>mgm./ 100 cc.</i>	<i>cc./hr.</i>	<i>cc./hr.</i>			<i>cc./am./ hr.</i>
I	49.5	1	125	281	11.4	10.0	2,465	2,340	12	5.2	406
		2	49	128	9.3	7.4	1,376	1,327	20	3.5	136
		3	39	107	7.4	6.0	1,446	1,407	19	2.4	154
		4	20	43	5.7	4.5	754	734	21	4.4	72
II	69.5	1	137	328	11.8	10.1	2,780	2,643	14	4.9	278
		2	73	146	9.6	8.2	1,521	1,448	14	4.9	150
		3	40	100	8.5	7.9	1,177	1,137	8	3.3	240
		4	27	66	8.0	7.4	825	798	8	3.0	158
III	51	1	35	81	21.8	19.1	372	337	12	9.4	59
		2	1.7	3.6	18.2	16.7	20	18	8	8.5	4.7
		3	1.8	3.3	17.4	16.0	19	17	8	9.5	4.5
IV		1	55	61	13.2	11.6	461	406	14	11.9	3.7*
		2	23	31	11.8	10.0	260	237	16	8.8	1.6*

* Blood flow in litres per hour for the whole kidney.

to 20 as the urine flow decreases from 125 to 49 cc. per hour and then remains stationary while the urine flow drops to less than half this value. The figures for plasma show a maximum of 25 per cent and a minimum of $1\frac{1}{2}$ per cent, the commonest range being between 10 and 15 per cent as in whole blood. The trend is for decreased diuresis to be accompanied by decreased filtration (e.g., expt. 2, table 2), but this is not always the case. In experiment 4 the rate of urine flow decreases from 20 to 6 cc. per hour while the percentage filtered, which changes 14 to 15 per cent, shows no indication of a drop. In experiment 1 the rate of urine formation doubles (22 to 45 cc. per hour) while the percentage filtration is unaltered.

To sum up, it may be said that although in many cases, probably the

majority, the height of diuresis is associated with increased filtration, this is by no means always the case.

The second factor, that of reabsorption in the tubules, can be represented in two ways, of which the more obvious, viz., the percentage reabsorbed, is perhaps not so useful as the percentage not reabsorbed; since variations are much more appreciable with the latter figures. For example, at a constant rate of filtration a decrease in the percentage absorbed from 95 to 90 per cent doubles the amount not reabsorbed, i.e., the urine excreted. Cushny attributes the greater duration of sulphate diuresis to the presence of sulphate in the tubules preventing as complete reabsorption as in its absence.

TABLE 2
Analyses of plasma

EXPERIMENT NUMBER	WEIGHT OF KIDNEY	SAMPLE NUMBER	URINE VOLUME	URINARY CREATININE	PLASMA CREATININE		FILTRATE	REABSORBED	PERCENT FILTRATED	PERCENT NOT REABSORBED	BLOOD FLOW
					Arterial	Renal volume					
	grams		cc./hr.	mgm./hr.	mgm./100 cc.	mgm./100 cc.	cc./hr.	cc./hr.			cc./gm./hr.
I	53	1	22	47	12.0	9.4	392	370	22	5.6	34
		2	45	72	9.5	7.5	758	713	21	5.9	68
		3	30	57	8.8	7.4	648	618	16	4.6	77
		4	16	40	8.3	7.1	482	466	14	3.3	63
II	37.8	1	64	83	25.7	21.1	323	259	17	19.8	48
		2	15	29	20.8	19.8	139	124	4.8	10.8	77
		3	5.5	5.7	19.2	19.0	29.7	24.2	1.5	18.6	75
III	45	1	98	145	14.0	10.5	1,036	938	25	9.5	92
		2	46	59	10.0	8.5	590	531	15	7.8	87
		3	17	20	9.2	8.0	217	206	13	5.1	38
IV	35	1	71	145	33.3	27.3	436	365	18	16.4	69
		2	9.7	22	25.0	24.0	88	78	4	11.0	63
		3	20	23	21.4	18.5	108	88	14	18.5	23
		4	6	8	20.7	17.6	48	42	15	12.5	7

In three of the experiments with whole blood the percentage not reabsorbed decreases with decreasing urine flow; in experiment 2 this is not the case. The experiments with plasma show much the same picture, but an exception may be pointed out in experiment 2 where the percentage not reabsorbed first falls and then rises with decreasing flow of urine. This phase would doubtless be clearer after a comparison with chloride diuresis (a study it is hoped to make). One can at least say that in nearly all cases increased reabsorption accompanies a decrease in urine flow.

The third factor is the blood flow through the kidney. The highest blood flow recorded is 406 cc. per gram per hour which is less than 7 cc. per gram per minute. This is definitely less than the highest value obtained

by Gibbs (1928) but is of the same order of magnitude as other determinations by him and previous authors. Increased blood flow may produce a larger volume of filtrate in two ways; viz., by an increase in the filtration pressure in each glomerulus; or the increase in both blood flow and that in volume of filtrate may be the expression of a greater number of active glomeruli. It is impossible to differentiate between these in this series of experiments although it is probable that the latter is the more important factor. In table 1 the urine flow and blood flow run together with two exceptions (expts. 1 and 2). In table 2 in rather more than half the samples this is true, but in the others increased urine flow is accompanied by decreased blood flow and vice versa. If one takes the volume of filtrate rather than the volume of urine for comparison, a similar statement may be made.

To sum up, it is impossible to assert that increased urine flow in sulphate diuresis is always accompanied by increased filtration, decreased reabsorption or increased blood flow. One or more of these factors is invariably present, but it is impossible at present to predict which of these is responsible for the greater formation of urine under the conditions of the experiment.

SUMMARY

An attempt is made to apply Cushny's theory to sodium sulphate diuresis. The blood flow and the amounts of fluid filtered in the glomeruli and reabsorbed in the tubules are calculated. Under the conditions of the experiment the diuresis is associated with one or more but not usually all of the following factors: increased blood flow; increased filtration; decreased reabsorption.

BIBLIOGRAPHY

- REHBERG, P. B. 1926. *Biochem. Journ.*, xx, 447.
OGDEN, E. 1930. *Proc. Soc. Exp. Biol. and Med.*, xxvii, 506.
BERGLUND, H. 1922. *Journ. Amer. Med. Assoc.*, lxxix, 1375.
UNDERHILL, S. W. F. 1923. *Brit. Journ. Exp. Path.*, iv, 87.
MAYRS, E. B. 1922. *Journ. Physiol.*, lvi, 120.
GIBBS, O. S. 1928. *Journ. Pharm.*, xxxiv, 276.

EXPERIMENTAL INTERFERENCE WITH RABBIT EMBRYOS IN THE EARLY STAGES OF THEIR DEVELOPMENT

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The relation of the corpus luteum to the survival of the early mammalian embryo was shown by Corner in 1928 in the first of his series of papers on the *Physiology of the Corpus Luteum*. He showed that if both ovaries of the rabbit are removed 14 to 18 hours after mating, the embryos will nevertheless develop normally to the early blastocyst stage; that normal development will occur and proliferation of the endometrium, when part of both ovaries is removed but one or more corpora lutea remain; and lastly that the removal of the corpora lutea by resection of the ovaries containing them or, in one case, the destruction of one ovary and the corpora lutea in the other by a galvanocautery, leads to the degeneration of the embryos during the first three days of their development. He suggested that, in the last cases cited, early death might be due to a different physiological condition in the tubes resulting from trauma.

Doctor Corner stated, in a personal communication, that it was sometimes impossible to find a trace of the developing eggs 3 to 4 days after cauterizing the corpora lutea. A few experiments were carried out at the Biological Laboratory at Cold Spring Harbor, in collaboration with one of us, in an effort to clear up the question of their early disappearance. Later the series was enlarged and a modified method added. Although the results do not have as broad a significance as we first thought that they might have, they are important as being the first record of the deliberate damage by experimental methods to early mammalian embryos.

METHOD AND RESULTS. The animals, common stock rabbits in good condition, were mated twice to one buck and insemination checked by the presence of spermatozoa in the vaginal smear. At periods varying from 15 to 21 hours after this, they were etherized and the ovaries exposed, with aseptic precautions, by flank incisions. With as little manipulation as possible, the corpora lutea were destroyed by a thermocautery. The incisions were closed and the animals were allowed to live for from 41 to 92 hours. They recovered from the operations satisfactorily and appeared in good condition, at the time that they were killed for autopsy by an overdose of ether. The entire reproductive tract was removed and rinsed

in salt solution and then the tubes or the uterine horns were washed out with normal saline according to the method used in the Carnegie Laboratories of Embryology and described by Corner in 1921. The embryos, when found, were compared as to stage of development with those described and illustrated by Gregory (1930). His large series of rabbits were killed at definite periods after mating and the embryos obtained by the above method.

In the 64 rabbits reported by Gregory, he recovered the eggs of 51 in normal condition. He failed in 8 cases to recover all of the ova that had been discharged, as indicated by the presence of recent corpora lutea. In

TABLE 1
Effect of cauterization of the corpora lutea on the young embryos

NUMBER	HOURS AFTER COITUS		CORPORA LUTEA	EMBRYOS	POSITION	CONDITION OF EMBRYOS	NORMAL FOR AGE
	Operation	Autopsy					
1	16	41	6	6	Tubes	3 unsegmented; 3 normal but slightly retarded, 1 6-cell and 2 7-cell	8-cell
13	21	47	8*	8	Tubes	6 normal 16-cell, 2 degenerating	16-cell
2	18	61	12	9	Tubes	Morula stage	Morula
5	18	69	7	4	Tubes	1 morula, 1 9-cell both abnormal, crystals in clear zone; 2 degenerating.	Morula
6	18	91	4	4	Uterus	2 normal blastula, 1 late morula, 1 degenerating	Blastula
3	21	92	8-10	8	Uterus	1 early blastula, 7 late morula. Crystals in clear zone, deformation of membrane	Blastula
4	15	96	4	1	Uterus	Degenerating blastocyst	Blastula

* One not cauterized.

4 animals one or more eggs were abnormal and in one some were retarded in development.

The essential data obtained from our experiments (table 1) are arranged according to the hours after coitus that the animals were killed. Though the series is not large, the fact is rather striking that in not a single instance were all of the discharged ova recovered in normal condition. The eggs of those killed soon after mating suffered slightly less damage. In the earliest, killed in 41 hours, half of the eggs were apparently normal for the period. In the second, killed in 47 hours, 6 of the 8 eggs which had been discharged were normal but the autopsy showed that one of the 8 corpora lutea had not been cauterized. There was one degenerating ovum, how-

ever, found in each tube. In the third, killed in 61 hours, 9 of the 12 ova were recovered in a normal condition but the other 3 were not found. Occasionally there were adhesions and in numbers 1 and 5 there was some hemorrhage about one tube. However, the injuries to the egg occurred when such conditions were not present.

It is possible that a toxic substance produced in the burned tissue was responsible for the damage to the young embryos. The absence of lutein tissue alone could not account for it, since development to the blastula stage will take place after both ovaries are removed, as quoted above.

Seven experiments were performed in order to test the toxicity hypothesis. Rabbits were mated and 19 to 23 hours later the tubes were

TABLE 2

Effect of cauterized tissue, other than the corpus luteum, on the young embryos

NUMBER	HOURS AFTER COITUS		CORPORA LUTEA	EMBRYOS	POSITION	CONDITION OF EMBRYOS	NORMAL FOR AGE
	Operation	Autopsy					
17	19	43.3	5	5	Tube	All 10 to 12 cell, crystals in clear zone of all, 2 embryos showed slight deformation	16-cell
16	21	44.5	7	7	Tube	6 8-cell crystals in clear zone and in embryos, 1 unsegmented	16-cell
14	19	48.5	11	1	Uterus	8-cell degenerating	16-cell in tube
12	20	67	11	2	Uterus	2 morula degenerating	Morula in tube
10	23	70.5	8	0			Morula
11	19	91	4	0			Blastula
9	21	95	7	2	Uterus	1 16-cell degenerating, 1 morula retarded	Blastula

exposed as in the first series (table 2). In order to lessen the manipulation, frequently no effort was made to actually see the ovaries. A piece about the size of a rabbit's ovary was cut from the edge of the incised flank muscle and fastened by a stitch to the fat mass in which the open end of the tube is imbedded. Then this muscle was cauterized, care being taken not to injure the tube, and the incision closed. In no. 17 the muscle was sewed to the inner surface of the flank muscle on the left side, near the tubal end, and then burned, while the right ovary was cauterized in two places. Both ovaries of no. 14 were treated as the right one in 17. In all of these great care was taken not to injure the corpora lutea. A microscopic examination of the ovaries was not made in any of these cases but on gross

inspection at autopsy the corpora lutea all appeared normal. The cauterized muscle was always in place.

It should be noted that, although there was an abundance of lutein tissue present, the ova were not recovered with regularity even in the retarded or degenerating state. In no. 14 one embryo was found in the uterus, 48.5 hours after ovulation. In another instance, no. 12, one was in the uterus in 67 hours. These two instances suggest that the tube was abnormally active, since Gregory gives 80 as the hours after coitus that embryos reach the uterus. In some cases the development was checked at the time of the operation, in others it continued for a few hours. The damage was more serious the longer the rabbit was allowed to live after the operation. These 7 experiments lend weight to the theory that a poisonous substance produced by the burned tissue injures the embryos, which must be very susceptible to chemical abnormalities in the environment.

SUMMARY

The results of 14 experiments are given, in which deliberate injury was done to young rabbit embryos by cauterizing the corpora lutea, or other parts of the ovary or by cauterized muscle fastened near the open end of the tube. The suggestion is made that the burned tissue produces a toxic substance.

BIBLIOGRAPHY

- CORNER, G. W. 1921. Carnegie Inst. Wash., Pub. no. 276, 117.
CORNER, G. W. 1928. This Journal, lxxxvi, 74.
GREGORY, P. W. 1930. Carnegie Inst. Wash., Pub. no. 407, 141.

THE STIMULATION OF MUSCLE RESPIRATION BY CARBON MONOXIDE

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It has been shown by Warburg (1927) that carbon monoxide inhibits the respiration of yeast. The completeness of inhibition depends upon the ratio of CO to O and is greater in the dark than in the light (Warburg and Negelein, 1928). Warburg has interpreted the results as indicating that CO combines with the iron-containing respiratory ferment in the same way that it combines with hemoglobin. It has been generally assumed that a similar inhibition of respiration would be found in all cells in CO but very few experiments are on record in which this effect was actually tried.

Warburg himself (1927) measured the aerobic glycolysis in thin sections of rat tissues (liver, chorion, retina, embryo, and Jensen's sarcoma) in light and dark with CO present. He found more aerobic glycolysis in the dark indicating a decreased oxygen consumption due to the CO. As he pointed out, it is difficult in mammalian tissues to get the ratio of CO to O large enough to cause inhibition without decreasing the oxygen supply so much that changes in respiration tend to be obscured. In addition to these observations Keilin (1927) has found inhibition of the indophenol oxidase from yeast and muscle by CO, Reid (1931) has observed inhibition of acetic acid oxidation by bacteria. Tamiya and Tanaka (1930) have reported that the metabolism of acetic acid bacteria is inhibited in the dark but revived in the light, and that the growth of *Aspergillus* (Tamiya, 1929) and the reduction of methylene blue by bacteria are inhibited by CO, (Tamiya, Hida and Tanaka, 1930). Tamiya (1929) states that the oxygen consumption of *Aspergillus Oryzae* (per gram) is decreased by CO but Warburg and Kubowitz (1929) found an inhibition. Fujita (1928) has observed also an inhibition of respiration in white blood corpuscles by CO as measured by glycolysis. Schmitt (1930) and Chang and Gerard (1931) have reported inhibition in frog nerve in the dark. The latter authors mention also inhibition in frog muscle mash "with occasional stimulating effects." Haldane (1927) has further observed toxicity of CO to moths which lack hemoglobin and to rats if the tension of CO is raised high enough to

more than saturate the hemoglobin even when the oxygen tension is high enough to ensure adequate oxygen supply in spite of the CO-hemoglobin. Reid (1931) has found inhibition of oxidation of acetic acid by bacteria with CO in the dark but not in the light.

On the other hand CO is without effect on the succinoxidase of muscle (Dixon, 1927) on the oxidation of erythrocytes in the presence of respiratory supplement (Michaelis and Salmon, 1930) on the autoxidation of SH (Dixon, 1928) on the anaerobic oxidation of acetic acid by bacteria in the presence of methylene blue or quinone (Reid, 1931), on peroxidase (Elliott and Sutter, 1932), on glucose oxidase from *Aspergillus* (Muller, 1929), on the reduction of methylene blue by liver extracts (Tamiya, Hida and Tanaka, 1930). Emerson (1927) has shown that CO does not inhibit the respiration of *Chlorella* except when the respiration of the plants is increased by immersing them in 1 per cent glucose or other substances. CO is in general non-toxic to invertebrates, microorganisms and plants (Haldane, 1927) and to bacteria (Fischer, Lieske and Winzer, 1931) but is reported toxic to molds (Seeländer). Its behavior is in general like that of nitrogen toward peripheral nerve (Krause, 1930), hens' eggs (Lallemand, 1929), erythrocytes (fragility tests, Mayers, Rivkin and Krasnow, 1930) and tissue cultures (Haggard, 1922).

From this brief review of the literature it would appear that CO does not uniformly inhibit respiration. It should be observed however that a negative result in this respect does not necessarily contradict Warburg's theory for it might merely mean that higher ratios of CO to O should have been used. In yeast Warburg (1926) has shown that the affinity of oxygen for the respiratory ferment is 7 to 14 times as great as that of CO.

Our attention was first directed to this problem by an experiment in which we were endeavoring to learn whether the increased metabolism of frog muscle which is caused by methylene blue would persist unchanged when the respiratory ferment was inhibited by CO. We eventually found that the increase due to methylene blue could be somewhat inhibited by CO but the experiment itself was of much more interest in that it showed a stimulating effect of carbon monoxide on the normal resting metabolism of the frog muscle. We might have been prepared to find no effect of CO in this experiment but there was no precedent for the finding of an actual increase and we were unable to believe the result until it had been repeated many times and controlled in all possible ways. We later obtained evidence that in frog muscle and certain other tissues CO is burned to CO₂ in amount sufficient to account in part at least and probably *in toto* for the stimulation observed.

METHOD. The oxygen consumption was measured in a differential volumeter (Fenn, 1927) with a capillary holding about 2.5 cu. mm. per cm. and bottles of about 10 cc. capacity. The volumeters were shaken in a

water bath at 22°C. at a rate of about 100 per minute with an excursion of about 3 cm. The bottles were of various sorts but mostly were fitted with two side arms at right angles to one another, one for NaOH and one for reagents which could be tipped on to the tissue as desired. Some were provided with stopcocks for the introduction of gases. When stop cocks were absent the CO mixtures were introduced through the one opening in the bottle and allowed to escape around the ground glass joint which was loosened slightly during the passage of the gas. Great accuracy in the tension of the gas was unnecessary in such cases. CO was prepared in the

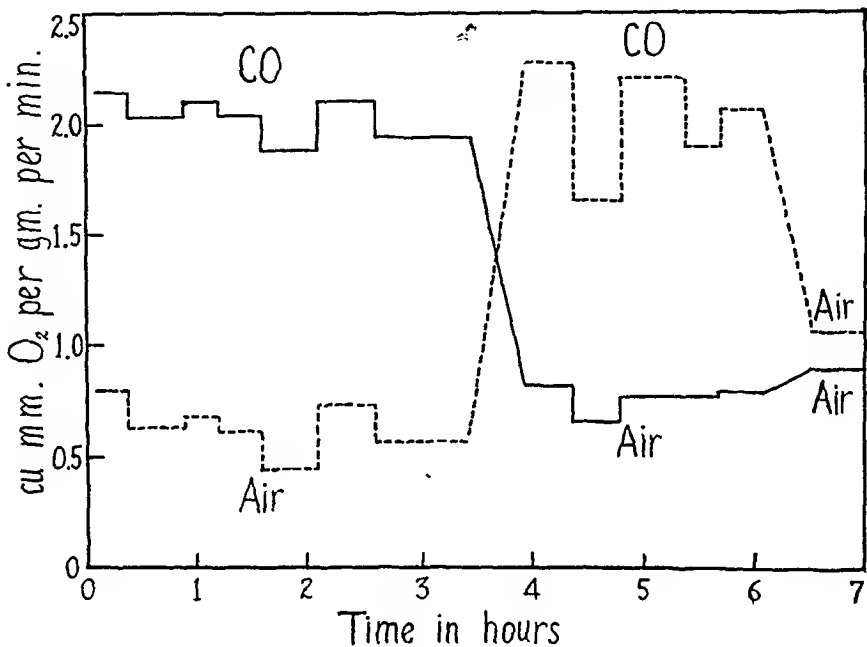


Fig. 1. Rates of gas absorption by two matched frog sartorius muscles in air and in 79 per cent CO, 21 per cent O₂. The muscles were in Ringer's solution in the respirometer in equilibrium with the gases which were changed as indicated. See table 1.

usual way by dropping formic acid on concentrated sulphuric acid or concentrated phosphoric acid. The gas was washed in strong KOH and in some cases through absorbent charcoal in addition in the hope of catching some impurity which might be responsible for the stimulation observed. The mixtures were prepared over water and were analyzed for CO₂ and O₂. When small CO concentrations were used the gas was measured accurately in a burette. The accuracy of the O₂ tensions was therefore carefully checked, the exactness of the CO concentration being of secondary importance.

EXPERIMENTAL RESULTS. The essential fact which we have to report

is illustrated in figure 1. Two sartorius muscles were dissected out from a frog and each muscle was placed in a respirometer in 1 cc. of Ringer's solution. NaOH was added in a side arm to absorb CO_2 . One respirometer contained air and the other contained a mixture of 79 per cent CO (approximately) and 21 per cent O. The resulting rates of respiration are plotted in figure 1 and it is apparent that the muscle in CO burns over twice as fast as the control muscle in air. After $3\frac{1}{2}$ hours the gas contents of the two respirometers are interchanged and the muscle which had been in air now burns in CO faster than its fellow in air. Finally both muscles are put into air and the rates of respiration are found to be approximately equal. The effects of CO are not apparently injurious and they are not persistent but quickly reversible.

A summary of seven experiments similar to that in figure 1 is given in table 1. The results show that the average rate of oxygen consumption

TABLE 1
Increased metabolism due to CO in normal frog muscle

IN AIR	IN 79 PER CENT CO, 21 PER CENT O ₂	PER CENT INCREASE
<i>cu. mm./gm./min.</i>	<i>cu. mm./gm./min.</i>	
0.71*	1.47	107
0.68 (fig. 1)	2.00	206
0.62	2.13	243
0.54	1.44	166
0.74	1.72	132
0.69	1.65	139
0.70	1.67	139
0.67	1.73	162

* Suspended in air. All others were in Ringer's solution.

in air was 0.67 cu. mm. O₂ per gram per minute. Replacing the nitrogen in the air by CO increased the rate to 1.73, an increase of 162 per cent. In making comparisons of this sort measurements in any mixture were continued long enough to reach a constant rate and the final rate attained was used in the tabulation. At least one hour was allowed after introducing a new gas mixture before rates were taken for comparison.

To study this surprising result in more detail a series of experiments was tried, similar in general to the one recorded in figure 1 but using concentrations of O₂ increasing from 0 to 100 per cent the remainder of the mixture being all CO or all nitrogen as the case may be. In each experiment matched sartorius muscles were used from a uniform batch of frogs and the gas mixtures were exchanged after a few hours. The two CO rates and the two control rates were then averaged together and the average percentage increase was thus determined. These averages are plotted

against the tension of oxygen in figure 2. The lower curve represents the control in nitrogen-oxygen mixtures and the upper curve the muscles in carbon monoxide-oxygen mixtures.

The curves coincide of course in 100 per cent oxygen and as the tension of oxygen is diminished there is no change in the oxygen consumption if nitrogen is used as a diluent until the oxygen tension becomes inadequate to supply oxygen by diffusion. The critical tension is in a 10-20 per cent oxygen mixture, thus showing experimentally that in more concentrated mixtures the muscles are well supplied. The sartorius muscles used for these experiments weighed about 70 mgm. When CO is used as a diluent instead of nitrogen the oxygen consumption (apparent) continues to in-

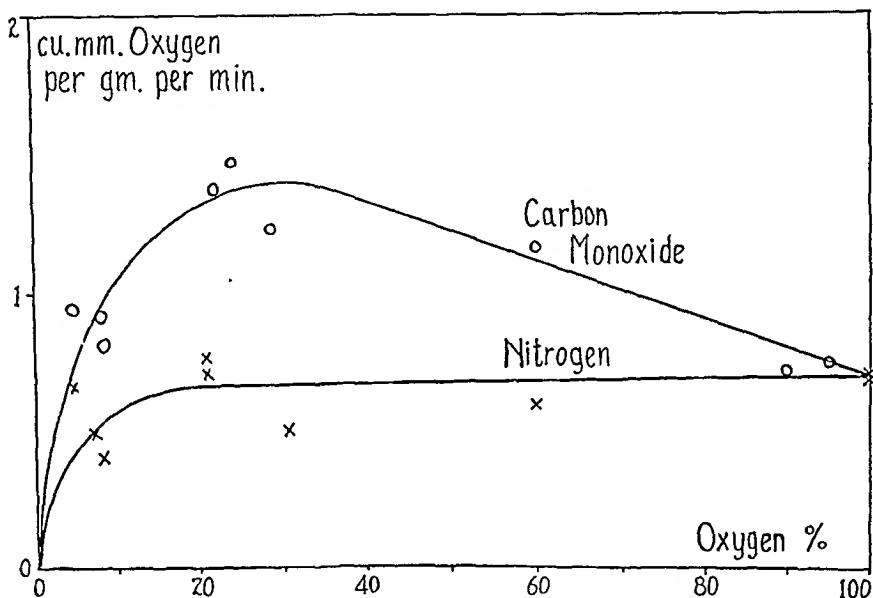


Fig. 2. Gas absorbed by a series of matched sartorius muscles, one in CO and one in N at varying tensions of oxygen.

crease until the oxygen tension becomes a limiting factor, when it must decrease to zero. We have ascertained by independent experiments that the oxygen consumption, or rather the volume diminution does actually go to zero when pure CO is used, i.e., CO is not absorbed in the absence of O₂. We have also tried control experiments in respirometers without tissue and have been able to show that the absorption of CO as formate by the KOH (Warburg, 1931) is not responsible for the greater rates in CO mixtures.

On the suspicion that this CO effect might be due to some error in our experimental procedure we repeated Warburg's experiment with CO and yeast and found a 73 per cent inhibition in the dark in 5 per cent O₂ and 95 per cent CO. Muscle evidently differs from yeast in this respect.

In order to find out whether this increase in the oxygen consumption in CO mixtures was dependent in any way upon the maintenance of normal contractility or irritability of the muscle we tried some experiments with rat muscle strips. The muscle is prepared by dissecting out with the scissors fine strips of the muscle consisting of individual fibres or bundles of fibres which are small enough, according to calculations, to provide sufficient oxygen by diffusion. In order to be sure of this point these muscle strips were kept at 22° during the experiment instead of 37°, their demand for O₂ being thereby diminished. Such muscle strips lose their irritability completely in Ringer's solution in about 15 minutes. In spite of this loss of irritability the metabolism is greater in the presence of CO than in a corresponding N₂-O₂ mixture. Five samples of rat muscle strips were prepared each weighing about 55 mgm. Each sample was placed in a separate respirometer in 1 cc. of Ringer's solution without phosphate buffers. Each respirometer was then filled with a gas mixture containing 20 per cent oxygen and varying amounts of CO up to 80 per cent, the remainder being nitrogen. The rate of oxygen consumption was then followed for over 5 hours. In the absence of CO the rate falls rapidly until it may practically disappear. In the presence of CO the rate is not much higher than in the control at the beginning of the experiment but the rate falls less rapidly and at the end of the first hour of the experiment the effect of the CO is very evident. Graphs of this experiment are shown in figure 3. Each curve represents the oxygen consumption in the different concentrations of CO at a given time, 1, 2, 3, and 5 hours, after the respirometers were put into the water bath. The effect of the CO becomes obvious in concentrations of 20 per cent or greater. One of the chief reasons for the fall in the metabolic rate in the absence of CO is the loss of substrate from the muscles by diffusion. The CO seems to serve to some extent as substitute although even in CO the rate decreases. Another similar experiment was tried with 10 per cent oxygen and Ringer's solution buffered with phosphate. In this case the oxygen consumption in the absence of CO did not fall so low but the increase in metabolism with increase in CO was evident, particularly in concentrations of 20 per cent or more. Lower concentrations were ineffective.

To test further the relation between contractility and the metabolism under CO we have soaked two matched muscles in isotonic KCl for 1 hour until they were quite non-irritable. Each was then put into a separate respirometer, one in approximately 21 per cent O plus 79 per cent CO and the other in air as in the experiment of figure 1. The rate in CO was 1.16 cu. mm. per gram per minute as compared to 0.82 cu. mm. in the control. As a further test the respirometers were removed from the bath after two hours and the gas mixtures were reversed so that the muscle which had been in CO was now put into N and the muscle which had been

in N was now put into CO, both being in 21 per cent O_2 as before. Now the CO muscle showed a rate of 1.66 as compared to 1.45 cu. mm. per gram per minute for the new control. Averaging together the two rates in CO and the two control rates we get figures of 1.41 and 1.13 cu. mm. per gram per minute. The metabolism is therefore increased 25 per cent by the CO. It is evident from this experiment that a completely non-irritable muscle produced by treatment with KCl will still respond to CO with increased metabolism. In one case a pair of spontaneously non-irritable muscles was obtained by omitting the use of Ringer's solution in dissection. In these muscles CO caused a 32 per cent increase in metabolic rate (table 2). The stimulation of metabolism by CO is therefore still present

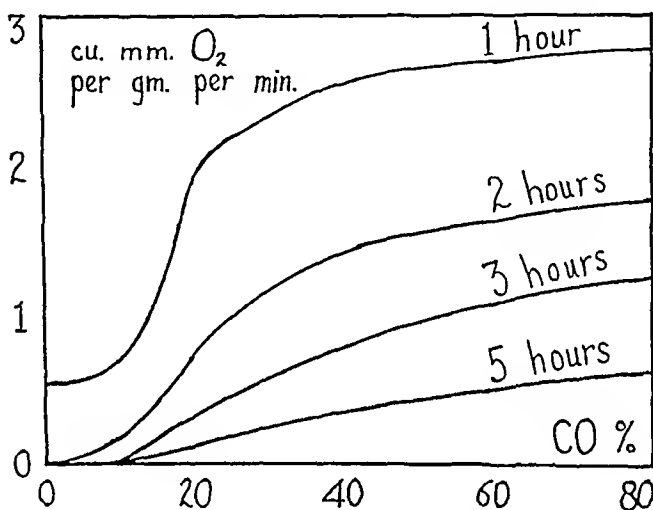


Fig. 3. Oxygen consumption of rat muscle strips in 20 per cent O_2 and increasing tensions of CO at different times (1-5 hrs.) after the start of the experiment. Temp. = 22°C.

in such muscles but it is distinctly less than the 162 per cent increase found in normal muscles.

A considerable number of other experiments of the type just mentioned have been carried out in the investigation of the subject. The results need not be given in detail but it will suffice for the purpose to indicate the percentage increase in metabolism which resulted, it being understood that the complete experiment in every case resembled that described above for KCl. The results are included in table 2.

For purposes of comparison table 2 includes the average figure for normal muscle as found in table 1. In frog muscle mash the increase in metabolism due to CO is only 30 to 60 per cent instead of 50 to 200 per cent as in normal muscles. It does not seem to make any difference whether

boiled muscle juice is added to the brei or not. Such addition tends to maintain the metabolic rate more constant during the experiment. Washing the minced muscle in water destroys its ability to consume oxygen but this ability is revived by the addition of sodium lactate as substrate. In such a preparation CO also serves to increase the metabolic rate 41 per cent. In one well washed muscle mash there was practically no metabolism either with or without CO and the metabolism was not revived by the addition of methylene blue 0.05 per cent or succinic acid 0.07 M.

TABLE 2
Increased metabolism due to CO

	AIR	79 PER CENT CO, 21 PER CENT O ₂	PER CENT INCREASE
	cu. mm./gm./ min.	cu. mm./gm./ min.	
1. Whole muscle			
a. Normal average.....	0.67	1.73	162
b. In KCl 0.9 per cent.....	1.13	1.41	25
c. Spontaneous non-irritable.....	1.07	1.41	32
d. In Na-bromacetate 0.03 per cent.....	0.65	1.41	119
2. Frog muscle mash			
a. In Ringer's.....	0.93	1.23	32
b. In Ringer's.....	0.61	0.86	41
c. Add boiled muscle juice.....	1.01	1.44	43
d. Add boiled muscle juice.....	0.76	1.25	64
e. Wash once in H ₂ O. Add Na-lactate..	0.27	0.39	41
f. Wash 3 times in H ₂ O.....	0-0.07	0-0.05	0
Add methylene blue and succinic acid			
3. Rat muscle			
a. Mash.....	0.47	0.71	51
b. Strips 37°C.....	6.29	7.20	14
c. Strips 22°C.....	1.73	2.68	55

Rat muscle strips both at 37°C. and at 22°C. and rat muscle mash also show a stimulation with CO.

The experiments of table 2 show that normal irritability is not necessary for the manifestation of the CO effect. This excess CO metabolism is either a true stimulation of the normal metabolism or a burning of something else. In either case this excess oxidation seems to be mediated by the normal oxidizing system. At any rate loss of materials from the particles of tissue in the muscle mash either by diffusion into the Ringer's solution or by washing with water result in a decrease of the excess CO metabolism as well as of the normal metabolism. Both fractions may be completely abolished by this means but usually the CO metabolism is less

affected so that the percentage increase due to CO becomes progressively larger as the experiment proceeds (fig. 3).

Both the normal and the excess CO metabolism are also decreased more or less equally by suspending the minced muscle in isotonic CaCl_2 . In one experiment of this type the substitution of CaCl_2 for NaCl decreased the metabolic rate in air from 1.48 to 0.09 cu. mm. per gram per minute while the excess rate due to CO was decreased from 0.77 to 0.10. Likewise KCN M/50 decreased both the normal metabolic rate and the excess CO rate of a sartorius muscle to about 10 per cent of the usual value. In KCN solution CO may still cause a considerable percentage increase in metabolism but the absolute increase is much decreased.

Early in the investigation we endeavored to test the possibility that the increased metabolism due to CO was caused by an increased rate of formation of lactic acid. We therefore soaked a muscle in 0.03 per cent sodium bromacetate solution which is sufficient according to the analyses of Hegnauer (appendix to Wright, 1932) on frogs similar to those used by us to abolish completely the formation of lactic acid. On testing the metabolism of such muscles with and without 80 per cent CO we found that the rate of oxygen consumption in the controls was 0.65 cu. mm. per gram per minute, this being increased to 1.41 by the addition of CO (see table 2). An increased lactic acid formation is therefore unnecessary for the CO effect.

As a further test of a possible correlation between the stimulating effect of carbon monoxide and a formation of lactic acid or possibly a breakdown of phospho-creatin we have measured the absorption and elimination of carbon dioxide in oxygen-free mixtures of carbon dioxide with nitrogen and with carbon monoxide. Such experiments in 20 per cent carbon dioxide mixtures have shown always an absorption of carbon dioxide indicating phospho-creatin breakdown, as shown by Meyerhof (1930). There was, however, no detectable difference between the nitrogen and the carbon monoxide mixtures in this respect. In 5 per cent carbon dioxide mixtures there was a preliminary absorption of carbon dioxide followed after two to four hours by a gradual elimination of carbon dioxide indicating the formation of lactic acid. Here again there was no perceptible difference between the muscles in carbon monoxide and those in nitrogen. Some difference may have occurred during the preliminary period of equilibration before it was possible to take readings but this seems improbable.

In some of these experiments oxygen was readmitted to the respirometer after 4 to 6 hours of anaërobiosis and measurements of oxygen consumption were made. These measurements showed that the prolonged stay in carbon monoxide had no lasting after-effects.

To study further this apparent independence of the lactic acid concen-

tration we have tried three experiments in which matched gastrocnemius muscles were put one into pure CO and the other into pure N. Both gases were freed from oxygen by passing them over hot copper. The CO was further washed in NaOH in order to remove the CO₂ formed by the reduction of the copper, and which may be present in considerable amounts. The muscles were suspended over Ringer's solution in glass stoppered tubes with stopcocks at each end. In two cases the muscles were left under such anaërobic conditions for 4 hours at 21–22°C. and were then analyzed for lactic acid by the Friedman, Cotonio and Schafer method. In a third experiment the muscles were at room temperature for 1.5 hours and at 4°C. for 15½ hours before analysis. The lactic acid contents found on analysis in table 3 show a slight increase of lactic acid in the presence of CO. In spite of this result it does not seem possible to explain the stimulating effect of CO by its slight effect on lactic acid formation. We therefore find these experiments on lactic acid formation difficult to interpret.

In one experiment an attempt was made to measure the pH of muscle in carbon monoxide as compared to the control muscles in the hope of explaining in this way the stimulating effect of carbon monoxide on respiration. For this purpose muscles were equilibrated at known tensions of carbon dioxide. The combined carbon dioxide was measured by tipping acid on the muscle and the pH was calculated in the usual way by the Henderson-Hasselbach equation. The muscle in carbon monoxide showed a pH of 6.94 in 3.65 per cent CO₂ and the control muscle showed a pH of 6.89 in 3.46 per cent CO₂.

Further preliminary experiments were tried to discover any possible change in irritability and contractility of muscles which could be attributed to carbon monoxide. For this purpose matched muscles were left, one in air and the other in a mixture of 21 per cent oxygen and 79 per cent carbon monoxide, and the threshold for electrical stimulation was followed. After 5 hours the control muscle showed a 38 per cent increase in irritability whereas the muscle in carbon monoxide showed an increase of 42 per cent. In other experiments the muscles were stimulated rhythmically after periods in carbon monoxide and air and a graphical record of contractions was obtained. In a number of such experiments we could establish no consistent difference between the muscle in carbon monoxide and the control muscle in air. It is possible that further more careful experiments might demonstrate a slight difference in favor of the control muscle. There is no contracture of any sort when a muscle is brought into a CO mixture.

In one experiment the ratio $\frac{Tl}{H}$ was measured for sartorius muscles stimulated by single break shocks by the myothermic methods of A. V.

Hill. T is the tension developed in grams. H is the initial heat in gram centimeters produced by a length of muscle of l cm. In 80 per cent CO and 20 per cent O_2 the ratio was 7.4–7.7 and 7.2 in air which is an insignificant difference. CO has therefore no considerable immediate effect upon the energy required for the development of tension.

The effect of light. The possible effect of light upon the CO metabolism is an important consideration in view of the work of Warburg and Negelein (1928). We have repeatedly measured the stimulating effect of CO upon respiration in 20 per cent O_2 mixtures on comparable muscles, one pair in ordinary daylight and the other pair with black bags tied around the respirometer bottles. Under such conditions we have failed to observe any constant difference attributable to light. Two experiments were tried to test more decisively the effect of light. Two matched muscles were used in each experiment one in CO- O_2 and one in N_2 - O_2 mixtures.

TABLE 3

*Anaerobic lactic acid formation
in CO and in N_2*

NUMBER	CO	N_2
	<i>per cent</i>	<i>per cent</i>
1	0.062	0.058
2	0.080	0.078
3	0.116	0.088

TABLE 4

*Respiratory quotient of frog sartorius
muscles*

IN AIR	IN 80 PER CENT CO, 20 PER CENT O_2
0.90	0.73
0.86	0.95
0.82	0.72
0.79	0.82
0.92	0.75
0.89	0.80
0.86 Average	0.79 Average

Twenty-one per cent oxygen was used in the first experiment. Measurements of O_2 consumption were made in alternating one-hour periods of light and dark. For dark periods the whole room was darkened and the water bath covered so far as was practicable. For light periods the shades were raised and two 100-Watt electric lights were turned on close to the respirometers. Readings were taken just before a dark period and 5 minutes after the light period began in order that no temperature differences or other similar artifacts could enter in. In each experiment 5 or 6 alternate periods were measured. The rate in the light was consistently slightly higher than the rate in the dark, the ratio of light to dark being on the average 1.08 in CO and 1.2 in air. The absolute rates were always much higher in CO, the increase being 2.64 times in the light and 2.94 times in the dark. It is evident that illumination of this intensity at least is not a factor of importance in comparison to the stimulating effect of CO. The stimulating effect of light in the presence of CO might have

been expected but it is surprising to find more stimulation by light in air than in the CO mixture. In all the experiments reported in this paper the respirometers were in ordinary daylight.

The respiratory quotient in CO. To learn more concerning the nature of the metabolism of muscles in the presence of carbon monoxide we made some measurements of the respiratory quotient. We used for this purpose the methods described by Dickens (1930) in which the carbon dioxide is caught in barium hydrate during the oxygen consumption measurement and then its amount determined by a subsequent acidification. The method need not be described in detail but the results are shown in table 4. Seven measurements of the respiratory quotient of normal sartorius muscles in air gave an average respiratory quotient of 0.869. This figure confirms a similar average obtained previously in a more extensive series (cf. Fenn, 1932). Similar muscles in 21 per cent oxygen and 79 per cent carbon monoxide showed a distinctly lower respiratory quotient of 0.794 average. If one excessively high value of 0.95 is omitted as probably erroneous the average is 0.76. Such a diminution in respiratory quotient could be explained by a burning of carbon monoxide to carbon dioxide. The theoretical R.Q. for this process would be 0.666. A simple calculation shows that a lowering of the R.Q. from 0.86 to 0.76 could be explained by an increase of 111 per cent in the rate of gas absorption, $\frac{1}{3}$ of which would be due to O_2 and $\frac{2}{3}$ due to CO. The extra CO_2 formed would be $\frac{2}{3} \times 111$ or 74 per cent. Thus
$$\frac{86 + 74}{100 + 111} = \frac{160}{211} = 0.76.$$
 This explanation is reasonable

because the average percentage increase in metabolic rate due to CO is as much or more than 111 per cent (table 1). Further confirmatory evidences will be presented in a later paper.

DISCUSSION. It is surprising that this stimulating effect of CO on muscle respiration should not have been discovered earlier. Apparently the effects of CO on muscle have not been previously investigated. In a later paper the effect of CO on other tissues will be described and it will then appear that skeletal and heart muscles are peculiar in this respect. Warburg (1927) tried the effect of CO on a number of rat tissues but he failed to try muscle. Keilin (1927) found that CO inhibited the indophenol oxidase of sheep's heart muscle but he did not try the intact muscle. Hence the experiments herein reported do not constitute a contradiction of any known facts nor in all probability of the familiar theory that CO inhibits respiration. The stimulating effect of CO seems to be in reality a phenomenon which is superimposed upon an inhibition due to CO. Evidence will be presented later to show that the phenomenon is really due to a burning of CO to CO_2 . The study of the conditions in the tissues under which this process is able to occur cannot fail to yield valuable information relative to the mechanism of tissue oxidations. The investigation is now being continued with this objective in mind.

SUMMARY

1. Substituting carbon monoxide for the nitrogen of the air surrounding a frog sartorius muscle increases the respiration usually 1.5 to 3 times.

2. A similar though smaller increase in metabolism is produced in the same way in muscle mash of frogs and rats, in frog muscles which are spontaneously non-irritable or non-irritable from isotonic KCl.

3. The increase is diminished but not abolished by loss of the irritability of the muscle; it persists when the formation of lactic acid is inhibited by sodium bromacetate treatment; it cannot be correlated with any changes in irritability or contractility of the muscle.

4. Both the normal respiration and the excess respiration due to CO are diminished to approximately the same extent by KCN, isotonic CaCl_2 and by loss of soluble material from the tissue by diffusion or water extraction.

5. Light has no significant effect upon the excess respiration due to CO.

6. The anaerobic acid-base changes of muscle, as followed by the CO_2 absorption or elimination in CO_2 atmospheres were not affected by the presence of CO in place of H or N. CO does not therefore affect significantly the formation of lactic acid or the breakdown of phosphocreatine although a slight increased anaerobic lactic acid content was found in gastrocnemius muscles in CO.

7. The tension developed in single shocks for a given amount of initial heat was not affected by CO.

8. The R.Q. of muscles was lowered from 0.87 to 0.76 by CO. This suggests a burning of CO to CO_2 .

BIBLIOGRAPHY

- CHANG, T. H. AND R. W. GERARD. 1931. *This Journal*, xcvii, 511.
 DICKENS, F. AND F. SIMER. 1930. *Biochem. Journ.*, xxiv, 905.
 DIXON, M. 1927. *Biochem. Journ.*, xxi, 1211.
 1928. *Biochem. Journ.*, xxii, 902.
 ELLIOTT, K. A. AND H. SUTTER. 1932. *Zeitschr. Physiol. Chem.*, ccv, 47.
 EMERSON, R. 1927. *Journ. Gen. Physiol.*, x, 469.
 FENN, W. O. 1927. *This Journal*, lxxxiv, 110.
 1932. In press. *Jour. Cell. and Comp. Physiol.*
 FISCHER, F., R. LIESKE AND K. WINZER. 1931. *Biochem. Zeitschr.*, ccxxxvi, 247.
 FUJITA, A. 1928. *Biochem. Zeitschr.*, cxvii, 189.
 HAGGARD, H. W. 1922. *This Journal*, lx, 244.
 HALDANE, J. B. S. 1927. *Biochem. Journ.*, xxi, 1068.
 KEILIN. 1927. *Nature*, cxix, 670.
 KRAUSE, F. 1930. *Acta psychiat. et. neurol.*, v, 473.
 LALLEMAND, S. 1929. *Bull. sci. pharmacol.*, xxxvi, 65.
 MAYERS, M., H. RIVKIN AND F. KRASNOW. 1930. *Journ. Ind. Hyg.*, xii, 300.
 MEYERHOF, O. 1930. *Naturwissenschaften*, xviii, 330.
 MICHAELIS, L. AND K. SALMON. 1930. *Journ. Gen. Physiol.*, xiii, 683.
 MÜLLER, D. 1929. *Biochem. Zeitschr.*, ccxiii, 211.

- REID, A. 1931. *Biochem. Zeitschr.*, ccxlii, 159.
- SCIMITT, F. O. 1930. *This Journal*, xcv, 650.
- SEELÄNDER, K. *Bot. Centralb. Beihefte. Abt. 1*, xxiv, 357.
- TAMIYA, H. 1929. *Acta Phytochimica*, iv, 227.
- TAMIYA, H. AND K. TANAKA. 1930. *Acta Phytochimica*, v, 167.
- TAMIYA, H., T. HIDA AND K. TANAKA. *Acta Phytochimica*, v, 119.
- WARBURG, O. 1926. *Biochem. Zeitschr.*, clxxvii, 471.
1927. *Biochem. Zeitschr.*, clxxxix, 354.
- WARBURG, O. UND E. NEGELEIN. 1928. *Biochem. Zeitschr.*, exciii, 334.
- WARBURG, O. AND F. KUBOWITZ. 1929. *Biochem. Zeitschr.*, cexiv, 24.
- WARBURG, O. 1931. *Biochem. Zeitschr.*, ccxlii, 174.
- WRIGHT, C. I. 1932. *Journ. Cell. and Comp. Physiol.*, i. Appendix by HEGNAUER, p. 236.

THE BURNING OF CARBON MONOXIDE BY HEART AND SKELETAL MUSCLE

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In a previous paper (1932) we have described experiments indicating that in an intact frog muscle the metabolism is increased between 2 and 3 times when the air surrounding the muscle is replaced by a mixture of 21 per cent O_2 and 79 per cent CO. The purpose of the present paper is to extend these observations to other tissues of both cold and warm-blooded animals and to show that this increased metabolism is not merely an increase in the normal metabolism but rather a CO metabolism in which CO is burned to CO_2 by the consumption of oxygen. The important fact emerges from these experiments that it is only in muscle tissue, either heart or skeletal, that this stimulation of metabolism or the burning of CO can take place to any important extent. The significance of this fact is not at present clear.

SERIES I. THE EFFECT OF CO ON DIFFERENT TISSUES. *Method:* The general technique employed for the oxygen consumption measurements of series I has already been described. For the particular experiments herein reported it may be stated that two differential volumeters are used in which are placed two comparable pieces of tissue. In one respirometer is put a mixture of O_2 and CO containing 21 per cent O_2 and, in the other, air. The oxygen tension is therefore the same in both and is adequate to meet the demands of the tissue. Oxygen consumption measurements are then made for 2 to 3 hours after which the gases in the two respirometers are interchanged. The oxygen consumption rates are again measured for a similar period of time. At least 1 hour is allowed before the final rates are obtained which are used in tabulating the results. In muscle there seems to be no after effect due to the period of CO so it seems permissible to average together the two rates so obtained in CO for comparison with the two rates obtained in air. Thus instrumental errors and random differences between the two samples are ruled out. The experiments were all carried on at 22°C. on frog, turtle, and rat tissues. The frogs were *Rana pipiens* or *R. clamitans* and the turtles mostly *Pseudomys elegans*.

Results. By means of the technique described we have made measure-

ments of the gaseous metabolism in a considerable number of different tissues of rats and frogs under the influence of CO. The results are included in table 1. The rates are given in cubic millimeters of gas per gram of fresh tissue per minute. Average figures for thin strips of rat muscle and for intact frog skeletal muscle are included from the previous paper for comparison. It is evident that in skeletal and heart muscle the effect of CO in the light is to cause an increase of 30 to 160 per cent in the metabolism. In other tissues there may be a small increase as in rat tumors, rat liver, frog liver and to some extent nerve, kidney and testis. In frog skin there seems to be a slight diminution even in 80 per cent CO in the light but in 97.6 per cent CO in the dark the inhibition may be as much as 40 per cent. Likewise in nerve in the dark we have one experiment which shows definite inhibition.

Some of these comparisons have been made in ordinary daylight and some in darkness produced by fastening black bags around the experimental bottles. Experiments done in the dark are indicated by a (d) in table 1. In most of these experiments we have not observed any difference which could be safely attributed to the light, and the light and dark experiments have therefore been averaged together. Probably any effect of the light was less than the experimental error.

SERIES II. THE BURNING OF CO. The proof that this increased metabolism is due to the burning of CO depends upon simultaneous measurements of the total diminution in volume, as measured on the differential volumeter, with the oxygen which disappears, as determined by actual gas analysis of the contents of the respirometer before and after a long period of respiration.

Method. The apparatus used for this purpose is shown in figure 1. It is essentially a differential volumeter with rather large bottles holding 32 cc. and fitted with a special stopper for the sampling of the gas and with a special stopcock at the bottom of the bottle for the introduction of mercury. Through this three-way cock mercury is introduced from the leveling bulb when the gas is withdrawn from the respirometer at the end of the experiment. A vertical graduated 1 cc. pipette is also sealed to the third opening on this cock so that small amounts of mercury may be admitted from the pipette at intervals during the experiment. This is done as often as the index drop is drawn to the end of the capillary tube connecting the two bottles of the respirometer. The drop is moved back again in this way every 10 or 20 minutes throughout the day depending upon the amount of tissue in the respirometer bottle. The mercury is thus in direct contact with the Ringer's solution containing the tissues but does not appear to have any injurious effect. The central well in the bottle contains M/1 NaOH for the absorption of CO₂. We have used 1 to 3 grams of tissue in 3 cc. of Ringer's solution in the bottle. This is sufficient to cause a

disappearance of 2 to 4 per cent of the total initial gas contained in the respiration bottle during the course of 5 or 6 hours.

When CO was used about 2 liters of the gas mixture were passed through the respirometer bottle, emerging at the stopper, where the last 100 cc.

TABLE 1
The effect of CO on the gaseous metabolism of different tissues

	CO-O ₂	AIR	PER CENT INCREASE	AVERAGE PER CENT INCREASE		CO-O ₂	AIR	PER CENT INCREASE	AVERAGE PER CENT INCREASE
	cu.mm. per gm. per min.					cu.mm. per gm. per min.			
Rat muscle.	—	—	—	55	Frog muscle.	—	—	—	160
Rat heart. ... (d)	3.68	3.02	22		Frog heart.	2.33	1.76	33	
Rat heart.	4.33	3.30	31		Frog heart.	2.94	1.35	118	
Rat heart.	6.42	4.81	34		Frog heart.	2.90	1.12	159	
Rat heart.	4.58	3.40	35		Frog heart.	1.96	0.90	113	106
Rat heart.	2.11	1.36	55	35	Turtle heart... (d)	3.69	1.83	102	
Rat liver. (d)	4.85	4.70	3		Turtle heart.	3.02	1.85	63	83
Rat liver. (d)	5.75	5.05	14		Frog liver†. ... (d)	2.08	1.72	21	
Rat liver. (d)	5.70	4.60	24	14	Frog liver.	2.53	1.96	29	
Rat kidney. ... (d)	11.29	11.37	—1		Frog liver.	2.56	2.41	6	
Rat kidney. ... (d)	10.90	9.80	11		Frog liver.	2.46	2.26	9	16
Rat kidney.	10.52	10.30	2		Frog kidney.	2.79	2.63	6	
Rat kidney.	11.20	10.00	12		Frog kidney.	2.69	2.42	11	
Rat kidney.	9.75	9.71	0		Frog kidney.	3.23	2.92	11	9
Rat kidney.	9.91	9.60	3	5	Frog nerve.	0.84	0.70	20	
Rat spleen.	3.87	3.72	4		Frog nerve.	0.84	0.72	16	18
Rat spleen. ... (d)	3.90	3.55	9	7	Frog nerve. ... (d)	0.65	0.73	—12**	
Rat spinal cord...	2.57	2.46	4		Frog skin.	3.45	3.55	—3	
Rat spinal cord...	2.46	2.54	—3	0	Frog skin.	2.82	3.10	—9	
Rat testis ... (d)	1.77	1.60	10		Frog skin.	1.77	2.28	—29	—14
Rat testis.	1.77	1.54	13	12	Frog skin. (d)	0.99	2.16	—54*	
Rat tumor. ... (d)	1.12	0.86	30		Frog skin. (d)	1.08	1.67	—35*	
Rat tumor.	1.87	1.53	22		Frog stomach.	0.75	0.77	—3**	
Rat tumor. ... (d)	2.75	2.53	9		Frog stomach.	1.83	1.78	+3	
Rat tumor.	2.75	2.44	13	19	Frog stomach.	1.81	1.93	—6	—2
					Yeast. (d)	0.3	1.16	—74	—74

The CO-O₂ mixture consisted of approximately 21 per cent O₂ and 79 per cent CO except where otherwise noted.

(d) = done in darkness.

* 2.4 per cent O₂.

** Approximately 10 per cent O₂ in both.

† The first 2 experiments with frog liver were done with large pieces and the last two with thin slices. The greater injury may cause less CO burning.

were caught over mercury in a sampling tube for analysis. The final oxygen content was determined at the end of the experiment, usually by withdrawing the gas directly into the analysis burette of a Henderson-Haldane gas analyzer. The analyzer was checked by analyzing room air before each experiment except in some of the earlier experiments. Two analyses were made of the gas in the respirometer at the beginning of the experiment and two at the end. In most of the experiments a second sample was taken for analysis at the end of the observation period after the first sample had been analyzed.

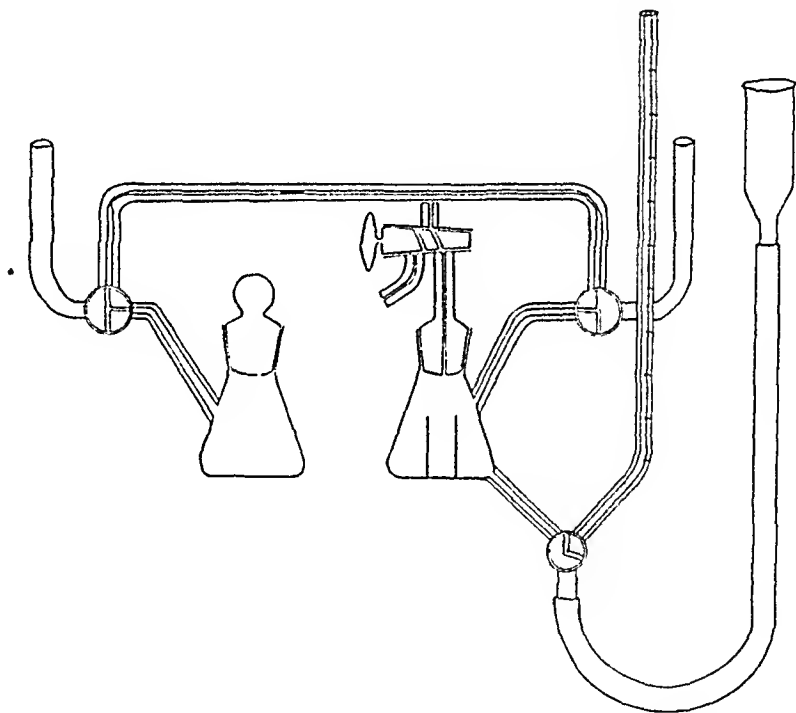


Fig. 1. Apparatus used for simultaneous measurement of total gas absorbed by volume and oxygen consumed by gas analysis.

The respiration period was calculated to the time of first sampling beginning at the time the CO mixture stopped passing. Since the respirometer was placed in the bath before the CO mixture was introduced equilibrium could be established rapidly after stopping the flow of gas and volume measurements could be begun within 2 to 4 minutes. The total volume diminution is measured directly on the vertical 1 cc. pipette with the addition of two short extrapolations at the beginning and end of the experimental period and a correction to take account of the position of the drop on the capillary tube.

The calculation of the amount of oxygen consumed during the experiment is made by the following equation

$$aV - x = b(V - x) \text{ whence } x = \frac{(a - b)V}{1 - b}$$

where V is the volume of the experimental bottle, a and b are the percentage compositions of the gas in oxygen at the beginning and the end respectively of the experimental period, and x is the volume of gas consumed (saturated with water vapor in equilibrium with the Ringer's solution in the respirometer at the temperature of the bath and the barometric pressure which obtained when the experiment was begun). In measuring V we included the narrow tubing leading from the experimental bottle up to the stopcock in the stopper and up to the bend in the index capillary. The total volume diminution as measured on the vertical 1 cc. pipette also refers to the moist gas as it exists in the respirometer bottle. The results have been so expressed in the tables without further correction to standard conditions.

Results. The observations of series II made by the method just described are collected in table 2. The 4th and 5th columns show the oxygen consumed as measured by gas analysis, calculations being made from the results of the analysis of the first and second samples respectively. The 6th column gives the total gas absorbed during the same period of time as measured by the amount of mercury which had to be introduced into the apparatus to keep the index drop in position. This total gas usually exceeds the oxygen consumed (4th column) by a certain amount which is shown in the 7th column expressed in percent of the oxygen. This represents the amount of CO which is consumed. Column 3 gives the average rate of oxygen consumption per gram per minute during the experimental period as calculated from the data of columns 1, 2 and 4.

The first 5 experiments of table 2 are controls showing the results obtained with frog muscle and turtle heart in air. The total gas absorbed agrees with the amount of oxygen consumed within 4 per cent. In the last 6 experiments using frog skin and liver and rat spleen and liver the total gaseous metabolism can be accounted for by the oxygen consumed within plus or minus 10 per cent. These are the tissues in which, according to table 1, there is little or no stimulation of the respiration by CO. In all the other experiments in table 2 the oxygen consumed fails to account for the total gas absorbed by amounts which are well outside the experimental error and range from 9 to 101 per cent. Thus in skeletal muscle and heart muscle there is some other gas absorbed besides oxygen. Since CO is the only other gas present it must be concluded that CO is absorbed. Since it has been shown in a previous paper that CO is not absorbed when O₂ is absent it must be concluded that some of the O₂ consumed is used for the

burning of the CO. The fact that this consumption of CO is found only in those tissues in which CO stimulates respiration, and then in amounts which are comparable in magnitude to the increase in metabolism observed, is strong evidence that all of the stimulation of respiration by CO is caused by the burning of CO to CO₂.

TABLE 2

Difference between total gas consumed and oxygen consumed, or the amount of CO burned

TISSUES	1	2	3	4	5	6	7
	WEIGHT OF TISSUE	TIME	O ₂ PER GM. PER MIN.	OXYGEN CONSUMED		TOTAL GAS USED	DIFFER- ENCE
				1st	2nd		
Controls in air							
	gms.	min.	cu. mm.	cc.	cc.	cc.	per cent
Frog muscles, intact.....	0.95	389	0.84	0.31	0.36	0.32	3.2
Frog muscles, cut up.....	3.55	411	0.76	1.10	1.14	1.14	2.7
Frog muscles, cut up.....	3.37	390	0.96	1.37	(1.36)	1.42	3.6
Frog muscles, cut up.....	4.13	393	0.72	1.16	1.23	1.20	3.4
Turtle heart strips.....	1.92	423	0.79	0.72	0.77	0.72	0
Experiments in 79 per cent CO + 21 per cent O ₂							
Frog muscle, intact.....	2.32	393	0.65	0.59		1.09	85.0
Frog muscle, intact.....	2.39	361	0.66	0.55		1.04	89.0
Frog muscle, intact.....	2.29	369	0.92	0.78		1.08	38.0
Frog muscle, intact.....	2.15	347	0.94	0.70		0.91	30.0
Frog muscle, cut up.....	3.20	394	0.70	0.88		1.11	26.0
Frog muscle, cut up.....	2.93	370	0.93	1.01	1.09	1.14	13.0
Frog muscle, cut up.....	3.73	256	0.69	0.66	0.71	0.78	18.0
Frog muscle, cut up.....	2.49	350	1.02	0.89	0.90	1.01	14.0
Frog muscle, cut up.....	3.48	371	0.83	1.07	1.09	1.30	22.0
Frog muscle, cut up.....	3.31	338	0.72	0.81	0.85	0.89	10.0
Frog muscle, cut up.....	2.51	320	1.02	0.82	1.00	0.98	20.0
Rat heart slices.....	1.08	296	2.72	0.87	0.92	0.98	13.0
Rat heart slices.....	1.24	301	3.65	1.36	1.45	1.67	23.0
Turtle heart strips.....	2.22	362	0.66	0.53	0.58	1.06	100.0
Turtle heart strips.....	1.17	395	0.76	0.35	0.45	0.64	83.0
Frog skin.....	3.63	299	0.80	0.87	0.92	0.81	-7.0
Frog skin.....	2.92	318	0.93	0.86	0.92	0.88	2.3
Frog liver.....	2.00	306	2.04	1.25	1.27	1.27	1.6
Frog liver.....	1.51	334	2.20	1.11	1.29	1.13	1.8
Rat liver.....	2.45	320	1.54	1.21	1.25	1.33	9.9
Rat spleen.....	1.04	287	3.65	1.09	1.15	0.99	-9.2

Most of the muscles used in the experiments of table 2 were not intact muscles but were simply pieces about the size of a sartorius muscle cut from the thigh of the frog. It was not realized at that time that the condition of the muscle would make any particular difference and it is difficult to get

enough tissue by any other means. These experiments on "cut up" muscles gave an average increase in metabolism due to CO combustion of only 17 per cent on the average. This was so much less than the 162 per cent found in intact muscles in the experiments of series I that we tried more experiments with intact muscles. This required the dissection of the sartorius, ileofibularis, and semitendinosus muscles from 4 frogs making 24 small muscles in all, each weighing 70 to 100 mgm. When these were used immediately after dissection the average rates of oxygen consumption were 0.92 and 0.94 and the percentage increases were 37 and 30 per cent respectively. Both of these rates are a little high for normal muscles. In a third experiment the 24 muscles were allowed to soak in 500 cc. of continuously aerated Ringer's solution in the cold room over night and were put into the respirometer in the morning. These muscles had a lower rate of oxygen consumption of 0.63 and a higher CO increase of 89 per cent and must be regarded as the most nearly normal of all those used in table 2. Another similar experiment showed an increase of 85%.

When 24 small muscles are put into 2 cc. of Ringer's solution immediately after dissection their rate of oxygen consumption never falls to normal levels. This is perhaps due to the K liberated from cells injured in dissection. Under such conditions CO does not seem to burn well.

Before leaving the discussion of table 2 attention should be drawn to column 5 which contains the figures obtained for the O_2 consumed from the analysis of the second sample withdrawn from the respirometer about 20 minutes after the first sample. During this interval the respirometer was not in the water bath and was not shaken so that it is impossible to say exactly how much more oxygen should have been consumed in this time. Furthermore after removing one sample for analysis the volume of gas remaining is much diminished so that further metabolism causes a greater percentage change in oxygen content than previously. For these reasons the second sample was used merely to confirm in an approximate way the results of the first sample. Calculation showed that the difference between the first and second samples was less than it should have been if respiration had continued at its previous rate. Probably the absence of shaking diminished the interchange between the solution and the gas.

In one of the control experiments (indicated in brackets, table 2) there is some obvious error for the oxygen consumed as calculated from the second sample (column 5) is less than that calculated from the first sample (column 4). It is possible that the error was in the analysis of the first sample rather than the second in which case the oxygen consumed would check even better with the total gas absorbed. In other cases in table 2 the difference between the oxygen consumed as calculated from the two samples is impossibly large. We have assumed, however, that the first sample is more likely to be correct and have used the first figure for com-

parisons. In the experiments on intact frog muscles only one sample was taken; this one sample was large enough for duplicate analyses. The figures obtained by this method are correspondingly more accurate. It is regrettable that the same procedure was not followed in all the experiments.

By way of summary a comparison is made in table 3 of the experiments series I and series II. In series I the total gas absorption only is measured on two samples, one in CO-O and the other in N-O mixtures, the differences giving the percentage increase. In the second series the oxygen consumed as measured by gas analysis is compared with the total gas absorbed the difference, expressed in percent, being CO consumed. The parallelism, while by no means exact, is good enough to be quite impressive. Discrepancies are due to variations in the condition of the tissue and to changes in the environment which determine whether CO can be burned or not.

TABLE 3

Comparison showing that the amount of CO consumed is sufficient to account for the stimulation of metabolism caused by CO

	INCREASE IN GAS CONSUMED IN CO— SERIES I	CO CONSUMED IN PER CENT OF O ₂ — SERIES II
	<i>per cent</i>	<i>per cent</i>
Skeletal muscle, intact.....	162	90
Skeletal muscle, cut up (av.).....	44	17
Cold blooded heart.....	98	92
Rat heart.....	35	18
Frog skin.....	-14	-2
Frog liver.....	16	+2
Rat liver.....	14	+10
Rat spleen.....	7	-9

That this is true for muscle has already been indicated. In liver it has appeared that large relatively uninjured pieces burn CO better than thin slices (table 1). Further work is necessary, however, before conclusions can be drawn for tissues other than muscles. In considering table 3 it should be remembered that the percentage increase in total gas absorption should be one-third larger than the CO consumed because one extra mol of oxygen must be absorbed to burn each mol of CO. From table 3 it may be concluded that the increased gas exchange in CO mixtures is due to the burning of CO.

DISCUSSION. One of the earliest theories of carbon monoxide poisoning suggested that CO acts in the body as it acts toward metals, i.e., as a reducing agent taking oxygen away from the blood and becoming itself oxidized and liberating CO₂ and heat in toxic quantities (Chénot, 1854).

In spite of the obvious error in this theory it now appears that this oxidation of CO can in fact occur in the mammalian body but it is doubtful whether this process is of any significance in actual cases of CO poisoning where the concentration of CO is so small in comparison with that used in these experiments. The oxidation of CO to CO₂ is also well known among the bacteria some of which (*Carboxydomonas*) derive their energy from this reaction. More recently it has been shown by Negelein (1931) that CO can be burned to CO₂ by hemin in $\frac{m}{1}$ NaOH if the O tension is low enough to leave the Fe only partially oxidized. Presumably therefore Warburg's iron-containing respiratory ferment is responsible for the oxidation of CO in tissues. This is confirmed by some observations previously recorded showing that both the normal metabolism and the CO metabolism are similarly affected by KCN, CaCl₂, and water extraction. It is possible that the increase in metabolism in *Chlorella* by CO as described by Emerson (1927) may also be due to a burning of CO by these plant cells.

SUMMARY

1. The effect of CO upon the gaseous metabolism of a variety of tissues has been studied in rats and frogs. Marked increases of 160 and 98 per cent are found in skeletal muscle and heart muscle respectively. Other tissues show smaller and less constant increases (liver, Jensen rat sarcoma, spleen, nerve, testis, cord, blood) or inhibition (frog skin).

2. By means of simultaneous determinations of total gas absorbed and of oxygen consumed (by gas analysis) it is shown that the increased gas absorption observed in CO is due to a burning of CO to CO₂ which likewise occurs to an appreciable extent only in heart and skeletal muscle.

BIBLIOGRAPHY

- FENN, W. O. AND D. M. COBB. 1932. This Journal, cii, 379.
CHÉNOT, A. 1854. C. R. Acad. d. Sci., xxxviii, 735 (see C. BERNARD-LÉCONS sur les anesthésiques et sur l'asphyxie, p. 404, Paris 1875).
NEGELEIN, E. 1931. Biochem. Zeitschr., ccxliii, 386.
EMERSON, R. 1927. Journ. Gen. Physiol., x, 469.

THE POSTERIOR PITUITARY HORMONE IN METABOLISM

III. THE EFFECT OF PITRESSIN AND PITUITRIN UPON THE LIPOID DISTRIBUTION

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Coope (1925) and Chamberlain (1925) observed marked changes in the contents of liver fatty acids in rabbits after pituitrin administration. Himwich and co-workers (1930a, 1930b) found decreases in the plasma fat of dogs dosed with pituitrin, pitocin, or pitressin. That a more complete picture might be presented, fat determinations were included in the carbohydrate studies made by the authors on rabbits dosed with pitressin or pituitrin (Bischoff and Long, 1931). In this series there were three groups of experiments—the first group of seven medullary adrenalectomized rabbits had been studied for varying lengths of time. The second group was deglycogenated by a dose of synthalin previous to the experiment. The third group had been standardized for insulin dosage. The doses of pituitrin were not as large nor the interval before death as long as the dosage and time interval in Coope and Chamberlain's experiments. Tissue analyses were confined to the liver and muscle. While such analyses in themselves might give valuable information as to the mobilization and utilization of fat, a knowledge of what happened to the sum total of fat in the body was necessary before definite conclusions might be drawn. Since the rabbit does not lend itself readily to such determinations, the study was continued upon mice.

EXPERIMENTAL. *Rabbit.* The moisture and fat content was determined in the liver and muscle of rabbits under the varying conditions described above. The sacrolumbalis muscle was excised from a freshly killed rabbit, freed from surrounding fat and connective tissue, cut into narrow strips and dried in an oven at 85° to 90°C. until a constant weight was attained. The dried muscle was then ground and samples weighed for ether extraction by the Soxhlet method. The whole liver minus the gall bladder and obvious connective tissue was dried and samples taken for Soxhlet extraction. Substances other than fats are obtained by ether extraction but the results obtained for the same tissues should be comparable. In a few experiments blood for fatty acid and cholesterol determina-

tions was taken before and after the substance being studied was administered. The fatty acids were determined by Bloor's method (1928) and since they include cholesterol, the latter was determined also by the method of Bloor, Pelkan and Allen (1922) and subtracted from the first value.

TABLE 1

The effect of pitressin and pituitrin upon the moisture and ether soluble content of muscle and liver of rabbits under various experimental conditions

RABBIT NUMBER	WEIGHT OF RABBIT	CONDITION OF ANIMAL	DOSAGE PER KILO-GRAM (SUBCUTANEOUSLY)	HOURS BEFORE KILLING	MUSCLE		LIVER		BLOOD (MOM. PER 100 cc.)			
					Moisture	Fat	Moisture	Fat	Choles-terol		Fatty acids	
									Before dosage	After dosage	Before dosage	After dosage
Group I												
3	1.93	Adrenalectomized	None		73.7	0.60	66.7	5.0		49		268
4	1.87	Adrenalectomized	None		79.6	0.30	69.4	4.6				
5	3.18	Adrenalectomized	None		75.1	0.23	71.2	3.6				
9	2.27	Adrenalectomized	15 units pituitrin	6	74.8	0.51	74.4	3.9	52	63	331	338
10	2.5	Adrenalectomized	15 units pitressin	6	78.1	0.58	73.1	4.2	77	56	243	195
1	2.16	Adrenalectomized	15 units pitressin	5	81.7	0.33	72.2	5.1	40	33	298	303
2	2.55	Adrenalectomized	15 units pitressin	5	77.7	0.36	71.3	4.6	46	46	176	185
Group II												
2	2.09	Deglycogenated by synthelin	3 gm. glucose at convulsion time	6	75.0	0.86	81.2	4.4				Plus cholesterol 190
3	2.1	Deglycogenated by synthelin	3 gm. glucose. 15 u. pitressin	6	75.0	0.87	85.7	3.2				
1	2.16	Deglycogenated by synthelin	3 gm. glucose. 15 u. pitressin. 1 mgm. adrenalin	6	74.2	0.82	77.8	4.6				Plus cholesterol 232
Group III												
14	1.81	Standardized for insulin	4 units insulin	1½	76.1	0.42	69.3	4.7		53		460
16	1.7	Standardized for insulin	3 units insulin	1½	74.9	0.47	68.8	5.4		68		435
15	2.0	Standardized for insulin	4 u. insulin. 15 u. pitressin	1½	74.1	0.52	69.5	5.9		47		456

Of the seven adrenalectomized rabbits, three were used as controls, one received 15 units of pituitrin, and three 15 units of pitressin per kilo subcutaneously 5 to 6 hours before sacrifice. Since the rabbits weighed around 2 to 3 kilos, the dosage of pitressin and pituitrin was under 2 cc. Coope and Chamberlain (1925) gave doses of 3 to 4 cc. of pituitrin but do not state the size of their rabbits. It will be noted from table 1 that the

ether soluble fraction of the liver and muscle varied as much or more for the controls as for the rabbits receiving pitressin and the one receiving pituitrin. The moisture content did not show any significant variation with the possible exception of the liver which showed an increased moisture content after pitressin.

In the second group of three rabbits, which had been given 6 mgm. of synthalin, 3 grams of glucose per kilo were administered when convulsions intervened, rabbit 2 acting as control, rabbit 3 receiving 15 units of pitressin per kilo, and rabbit 1, 15 units of pitressin per kilo, and 1 mgm. of adrenalin. The rabbits were sacrificed 5 hours later. It will be noted that the muscle fat content of the synthalin group ranges somewhat higher than that found for the other group. The ether soluble fats of the liver are within the average range.

In the third group, rabbits 14, 15 and 16 had received insulin over a period of several weeks until the smallest doses causing a convulsion were determined. On the day of the experiment, rabbit 14 received a sub-convulsive dose, rabbits 16 and 15 a convulsive dose, and in addition rabbit 15 received 15 units of pitressin which prevented a convulsion. All of the rabbits were killed at the time required for rabbit 16 to convulse, which was approximately $1\frac{1}{2}$ hours. The ether soluble liver and muscle fraction as well as the water content were within the normal variation.

The cholesterol and fatty acid values of the blood determined before and after dosage did not vary significantly in any of the groups of experiments.

Mice. Fat was determined on whole mice by a modification of Mottram's method. Male mice approximately 6 weeks of age with an average weight of 15 grams were used. Four controls and four dosed animals were run simultaneously so that mice of the same age and litter might be compared.

The mice were killed by a blow on the head, weighed and put into 20 cc. of 60 per cent KOH, heated in a boiling water bath until all except the bones were dissolved. Twenty cubic centimeters of 90 per cent alcohol were added and the heating continued for an hour. The contents of the flasks were washed into 300 cc. Kjeldahl flasks with hot water and cooled. Thirty-five cubic centimeters of 40 per cent H_2SO_4 were cautiously added, keeping the flask cooled under running water. After heating a few minutes in a water bath the flasks were again cooled in ice water. Fifty cubic centimeters of petroleum ether were added with a pipette and the flasks tightly stopped with tin-foil-covered corks moistened with glycerin. The flasks were then shaken some 30 times at intervals of a minute each time for about 10 seconds, filled with water so that the petroleum ether layer came up into the neck of the flask, then allowed to stand for an hour or more, giving the neck of the flask a sharp blow with the hand occasionally to facilitate the emulsion to separate. Usually a 40 cc. aliquot can be pipetted off into tared flasks and the ether evaporated. While the flask is still full of fumes, it is put into a vacuum desiccator, the remainder of the ether vapor being removed in this way. The weight became constant on the second or third day.

The results are tabulated in groups (table 2), the dosed mice with the controls, which were killed at the same time, and represented litter mates or mice of the same age. There was much variation between different groups of controls, so that each group of dosed mice must be compared with the controls killed at the same time. The first group of dosed mice received 0.25 unit of Parke-Davis' pitressin at 6 and 3 hours before killing, having fasted 24 hours in all. The variation among these mice was as great as their controls, and the means showed no significant difference. Another group received four doses of 0.25 unit every 3 hours during the last half

TABLE 2

Lipoid substances according to Moltram's method of whole mice after various doses of pitressin and of controls

	0.5 PRESSOR UNIT OF PITRESSIN*	CONTROL	1.0 PRESSOR UNIT OF PITRESSIN†	CONTROL	24 PRESSOR UNITS OF PITRESSIN†	CONTROL
	per cent	per cent	per cent	per cent	per cent	per cent
Group 1.....	3.3	2.8	2.1	3.2	11.6	
	3.0	2.8	2.1	4.1	6.0	
	4.1	2.9	2.1	4.0	10.8	
	4.1	3.1	2.1	3.0	11.6	
Group 2.....	3.0		6.5	4.8	7.0	
	3.3	2.8	6.6	4.1	5.1	5.3
	3.4	2.5	2.1	3.4	5.4	
			5.3	2.9		
Group 3.....	3.3		3.1		2.5	
	2.5	3.5	4.5		2.2	2.8
	4.0	3.1	1.8		2.1	
	5.0	4.0	2.8			
	3.5±0.21	3.1±0.15	3.4±0.44	3.7±0.21	6.4±1.6	

* Starved 24 hours, dosed 6 hours before killing.

† Starved 24 hours, dosed 12 hours before killing.

of their 24-hour fast, 1.0 pressor unit giving no more definite results. The variation was greater than for those receiving 0.5 unit. In fact, the results were paradoxical, some values falling below the lowest control range while others exceeded the highest values of the control range. The means of the dosed and control groups showed no significant difference.

To determine whether the dose given was pharmaceutical, blood sugars were determined for mice after giving them doses of pitressin. Since it was impossible to obtain enough blood from the tail to determine even micro sugars, the animal was sacrificed by cutting the throat. The blood was quickly collected with a micro pipette and put into the acid tungstate

solution. The value obtained by this procedure was compared with the value obtained for a composite sample taken from the tails of two or more mice. Values of 104 and 96 mgm. per 100 cc. were obtained on the composite sample as compared to 102 mgm. when collected from the throat. Fifteen minutes after a second dose of 0.25 unit of pitressin given 3 hours apart, the blood sugar was 190. Fifteen minutes after 1.0 unit had been given in four doses of 0.25 unit each every 3 hours, the value was 160 mgm., 30 minutes later 210 mgm., and after 45 minutes, 133 mgm.

The lethal dose of pitressin for the mouse was found to be around 30 pressor units, given in three divided doses 4 hours apart. If pitressin affects fat metabolism, the results should be exaggerated with an excessive dose. Accordingly, a sublethal dose of 24 pressor units was given in three doses of 8 units each over a period of 12 hours, after a 12-hour fasting period. At the end of the 24 hours, the mice were killed and subjected to analysis by the modified Mottram method. The first group showed very high results but unfortunately no control was run. The next group was high but the control was just as high, and the next group gave as low figures as had been obtained in other groups. The petroleum ether soluble content of the saponified mouse is apparently subject to a marked individual variation and values obtained without a control group are of little value. The mean for the lipoid substances of the whole group dosed with 24 units was 6.4 ± 1.6 per cent, which is higher than the mean for groups of lesser dosage. Only two controls were done for this group with values of 5.3 per cent and 2.8 per cent, giving a slightly higher average than the other groups.

DISCUSSION. Since pituitrin has found extensive clinical use in the treatment of obesity, evidence from animal experimentation that fat metabolism is affected by pituitrin has been sought. It must be admitted that convincing evidence is as yet entirely lacking. The work of Coope and Chamberlain (1925), which demonstrated an increase in liver fat, and the experiments of Raab (1926) and of Himwich and his co-workers (1930a, 1930b) on blood fat decreases, indicate changes in fat mobilization rather than fat utilization. The decrease in blood fat observed in the initial stages of pituitrin activity is at least in part explained by the hydration of the blood, a well-established pituitrin effect. Coope and Chamberlain are not inclined to regard the increases in liver fat observed by them as due to "fatty infiltration," although massive doses of pituitrin were used. The doses used in the present studies while less are nevertheless pharmaceutical as shown by the hyperglycemia, urination and diarrhea which followed each injection. Rony and Ching (1931) were unable to find any significant effect of pituitrin upon an alimentary lipemia. Our data show that in the rabbit pharmaceutical doses of pitressin did not affect the hepatic or peripheral fat deposits significantly. If there is mobilization or a change

in utilization, the fat content of the liver and muscles is held within certain limits.

Using Rubner's figures, the amount of fat oxidized by an 18 gm. mouse in a 4-hour period, assuming that fat was the only substance metabolized, would be 0.068 gram. On the basis of the fat analysis obtained in the present studies, the standard deviation of the mean of the fat content of a group of eight mice fasted 24 hours is ± 0.04 gram. The difference in the means of two series determined was twice this deviation. It is therefore apparent that profound changes in fat metabolism (100 per cent change) might actually take place, without detection, because of the factor of individual variation. The method (analysis of total fat content) is therefore eliminated as an attack to the problem of establishing the effect of the hormones (insulin, adrenalin, etc., as well as pituitrin) upon fat utilization over the active period of a single intravenous or subcutaneous dose.

In the present studies the active period was extended to 12 hours by repetition of dosage. On the basis of an eighteen gram mouse, the joint standard deviation of the mean (square root of the sum of the squares) for the control and dosed series equals ± 0.087 gram fat. Using the same assumption for the 12-hour period that was used for the 4-hour period, the maximum amount of fat that could be oxidized by the controls is 0.204 grams. A change in fat content corresponding to a 42 per cent change in metabolism would therefore be without significance.

SUMMARY

The moisture content, the ether soluble fats of the muscle and liver of rabbits determined under various conditions after the administration of pitressin, pituitrin and insulin did not vary significantly. The fatty acids and cholesterol values of the blood determined upon the same animals before and after dosage came within normal variations.

No significant changes in the total lipid content of mice were noted following pitressin administration over a wide range of dosage. The individual variation of the total lipid content was found to be of a magnitude so great that a 100 per cent change in fat metabolism over a 4-hour period would not affect the fat content significantly.

Doses of 0.5 unit and 1.0 unit of pitressin cause a hyperglycemia in mice when given in divided doses of 0.25 unit 3 hours apart.

The lethal dose of pitressin for mice was found to be around 30 pressor units when given in divided doses over a 12-hour period.

BIBLIOGRAPHY

- BISCHOFF, F. AND M. L. LONG. 1931. *This Journal*, xcvii, 215.
BLOOR, W. R. 1928. *Journ. Biol. Chem.*, lxxvii, 53.
BLOOR, W. R., K. F. PELKAN AND D. M. ALLEN. 1922. *Journ. Biol. Chem.*, lii, 191.
COOPE, R. 1925. *Journ. Physiol.*, lx, 92.

- COOPE, R. AND E. N. CHAMBERLAIN. 1925. *Journ. Physiol.*, lx, 69.
- HIMWICH, H. E. AND M. L. PETERMANN. 1930. *Proc. Soc. Exp. Biol. and Med.*, xxvii, 814.
- HIMWICH, H. E., F. W. HAYNES AND M. A. SPIERS. 1930. *Proc. Soc. Exp. Biol. and Med.*, xxviii, 332.
- RAAB, W. 1926. *Zeitschr. f. d. ges. exp. med.*, xlix, 179.
- RONY, H. R. AND T. T. CHING. 1931. *Endocrinol.*, xiv, 355.

THE FORMATION OF GLYCOGEN FOLLOWING PANCREATECTOMY

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The present study was undertaken to determine if the depancreatized organism has the ability to form glycogen in muscle and liver. Most, if not all, of the investigations that have been reported on the formation of glycogen have been done in the presence of complete diabetes of recently depancreatized or eviscerated animals. Objection is raised to this approach to the problem in that such animals are not necessarily in the diabetic state because of the short time since removal of the pancreas or because they are not in good physiologic condition. With this in mind, it was deemed advisable to allow the animals to recover completely from removal of the pancreas before attempting to induce the formation of glycogen in the liver and skeletal muscles. The conclusion that this process requires insulin is puzzling, even if much of the present experimental evidence is in favor of this view, since on discontinuing the administration of insulin to the depancreatized dog, the glycogen content of the skeletal muscles is not appreciably diminished.

In spite of the strides which have been made in the last decade on problems concerning carbohydrate metabolism, we must admit that even now we do not know the fundamental difference between the diabetic and the nondiabetic states. It is possible that this difference is quantitative rather than qualitative. It seemed possible to us that the animal with diabetes of maximal degree might retain the ability to form glycogen, provided proper conditions were established.

The conditions which seemed essential to our investigators were: 1, pancreas completely removed; 2, maintenance of the animal under insulin treatment until it was in good condition and the operative wound had healed, and 3, withdrawal of insulin for a period of five days before beginning the final part of the experiment. Although it cannot be stated that some insulin was not present at the end of five days after its withdrawal from the depancreatized animal, it is not practical to wait much longer because of the severity of the diabetes. The recent investigations of Best, Jephcott and Scott would indicate that animals under the conditions of those in our experiments would not have a significant amount of insulin.

PREVIOUS INVESTIGATIONS. Considerable data have been recorded in support of the belief that the organism with complete diabetes is incapable of forming glycogen, although a few investigators have published experiments contradictory to this view. Soskin (14) concluded that the dog with complete diabetes has little if any impairment in ability to oxidize carbohydrate. Chaikoff (4) determined the average glycogen content of the skeletal muscles in fasted, depancreatized dogs on the third and fifth days after withdrawal of insulin. He noted that the difference in the two determinations was not sufficient to account for the energy which had been expended during the time between the two estimations, and concluded that in order to supply this energy, glycogen must have been formed in the muscle, unless it can be assumed that other substances than glycogen can serve as the fuel for the production of energy in muscle.

Fisher and Lackey studied the glycogen content of the heart muscle, skeletal muscle and liver in normal dogs and in diabetic dogs on the same standard diet. The diabetic dogs did not receive insulin. It was found that the glycogen in the heart muscles of diabetic dogs was considerably higher than in normal dogs, and the converse was true of the skeletal muscles and liver. Cruickshank (6) also demonstrated an increase in the glycogen content of heart muscle following pancreatectomy.

Macleod and Markowitz (12) found that five days after the last administration of food or insulin to a depancreatized dog the skeletal muscles contained large amounts of glycogen. Macleod (10) (11) has shown that, although glycogen is always present in the skeletal muscles of depancreatized dogs, the injection of insulin effects a deposition of more glycogen in such muscles.

Choi, working on cats, found that glycogen does not form in muscle after evisceration regardless of the concentration of blood sugar. Best, Hoet and Marks (1) found that in the recently depancreatized cat the intravenous infusion of glucose is not followed by an increase in glycogen in muscles unless insulin is injected.

Markowitz, Mann and Bollman (13), working on recently depancreatized nephrectomized dogs, found it difficult to demonstrate an increase of glycogen in muscles subsequent to the injection of large quantities of glucose, although in some cases glycogen was undoubtedly formed. The objection to this, and to other similar work, is that insulin may have been stored in the tissues in sufficient quantities to effect the formation of glycogen. Fraser and Macleod endeavored to find some form of carbohydrate which would induce the formation of glycogen in muscle in the eviscerated preparation, but they were unsuccessful except for one experiment in which dihydroxyacetone was injected. Kermack, Lambie and Slater (9) also were able to demonstrate an increase of glycogen in muscles in recently eviscerated cats when this form of carbohydrate was injected. They also

observed an increase of glycogen in muscles following the infusion of glucose.

The most recent work on the subject is by Bodo, Tui and Farber (3). They studied the changes in glycogen in the liver following the injection of glucose in animals that had been depancreatized forty-eight hours previously. They found slight but definite increase of glycogen.

METHODS AND MATERIAL. The experiments were all performed on dogs. Operative procedures, including the taking of specimens of muscle were carried out under ether anesthesia. The pancreas was removed according to a method employed in our laboratories. The essentials of the method are: 1, to remove the organ by blunt dissection with an aneurysm needle; 2, to take care never to break the gland so that the danger of leaving bits of pancreatic tissue is reduced, and 3, never to ligate blood vessels. After removing the pancreas the duodenum was covered with omentum, and the abdomen was closed in the usual manner. After operation the dogs received intravenous injections of glucose twice daily for three days following pancreatectomy, but nothing by mouth during this interval. Subsequently they received a liquid diet of milk and syrup, and finally, on the fifth day a solid diet of ground meat and crackers, to which a little raw pancreas was added. Injections of insulin were begun on the day following operation.

After the abdominal wound had healed entirely, which usually required several weeks, food and insulin were withheld for five days before the actual experiment was begun. Glucose-nitrogen ratios were estimated to determine whether the animals were in a complete state of diabetes at the end of the fasting period, before beginning the actual experiment. Determinations of nitrogen were made according to the method described by Kjeldahl, and estimations of sugar in the urine were done by the method described by Benedict.

In all experiments control specimens were taken either from the same muscle or from the corresponding muscle of the opposite side of the body. That such a specimen serves as an adequate control has been shown in a previous paper. Determinations of the glycogen content were done by a modified Pflüger method. The injections of glucose were given by the intravenous route. After the animals had been killed careful search was made for remaining bits of pancreatic tissue throughout the abdominal cavity, special attention being paid to the walls of the intestine and gall bladder, as well as to the bed of the pancreas. Pancreatic remnants were not found in the experiments reported. The livers of all of the animals were characteristic of the liver of the depancreatized animal.

RESULTS. The detailed results of each experiment are given in the protocols. In summary they are as follows: fifteen experiments were performed on depancreatized dogs in which the glycogen of the liver and muscles was

determined before and after the intravenous infusion of glucose. In nine experiments glycogen formed in the liver in significant quantities, it was slightly increased in one experiment, and was diminished in five experiments. The glycogen in muscles was decreased in eight experiments; it was slightly increased in one experiment, and was significantly increased in five experiments.

EXPERIMENTAL DATA. *Experiment 1.* The weight of the dog was 7.5 kgm. The abdominal wound was completely healed one month following pancreatectomy. The first specimens of muscle and liver were secured at 9:02 to 9:25 a.m. From 10:00 until 11:58 a.m. 0.00075 gram of epinephrine for each kilogram of body weight per hour was injected intravenously continuously. The second specimens of muscle and liver were secured as 12:00 m. to 12:15 p.m. From 12:30 p.m. until 4:30 p.m. the dog received 2 grams of glucose intravenously for each kilogram of body weight per hour. The glucose-nitrogen ratio during the twelve hours prior to beginning the experiment was 2.2:1. The entire body of the animal shivered when the final specimens were taken. Estimations of glycogen were as follows:

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Liver.....	9:16 a.m.	0.093
Liver.....	12:10 p.m.	0.075
Liver.....	4:42 p.m.	0.068
Left sartorius.....	9:13 a.m.	0.222
Right sartorius.....	12:03 p.m.	0.080
Right sartorius.....	4:35 p.m.	0.032
Left quadriceps.....	9:14 a.m.	0.153
Right quadriceps.....	12:05 p.m.	0.090
Right quadriceps.....	4:31 p.m.	0.021
Left quadriceps.....	4:36 p.m.	0.024
Left gracilis.....	9:15 a.m.	0.085
Right gracilis.....	12:06 p.m.	0.071
Left gracilis.....	4:32 p.m.	0.032
Right gracilis.....	4:38 p.m.	0.026
Left adductor.....	9:17 a.m.	0.194
Right adductor.....	12:07 p.m.	0.124
Left adductor.....	4:34 p.m.	0.008
Right adductor.....	4:40 p.m.	0.100

Experiment 2. The weight of the dog was 7.5 kgm. The wound was entirely healed six weeks following pancreatectomy. From 9:00 to 11:00 a.m. 0.1 mgm. of epinephrine was injected intravenously for each kilogram of body weight per hour, and from 2:00 to 5:00 p.m. glucose was injected

intravenously at the rate of 2.0 grams for each kilogram of body weight per hour. The first specimens of muscle and liver were secured at 1:30 to 1:58 p.m. The second specimens were obtained after the animal had been killed. The glucose-nitrogen ratio during the twelve hour period prior to beginning the experiment was 2.8:1. Estimations of glycogen were as follows:

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Liver.....	1:48 p.m.	0.245
Liver.....	5:15 a.m.	0.206
Right sartorius.....	1:40 p.m.	0.018
Left sartorius.....	5:01 a.m.	0.002
Right quadriceps.....	1:41 p.m.	0.092
Left quadriceps.....	5:04 a.m.	0.001
Right gracilis.....	1:43 p.m.	0.003
Left gracilis.....	5:22 a.m.	0.001
Right adductor.....	1:44 p.m.	0.122
Left adductor.....	5:10 a.m.	0.002

Experiment 3. The weight of the dog was 8.5 kgm. The abdominal wound had healed six weeks subsequent to pancreatectomy. Epinephrine was not injected, but the animal was placed in the cold to promote glycogenolysis. The first specimens of muscle were secured at 2:20 to 2:45 p.m. The final specimens were removed after the animal had been killed. Glucose was not administered on the first day of the experiment, but from 9:00 a.m. on the second day until 5:30 a.m. on the third day the animal received 1.5 grams of glucose intravenously for each kilogram of body weight per hour. The glucose-nitrogen ratio prior to beginning the experiment was 2.7:1. Estimations of glycogen were as follows:

<i>Muscle</i>	<i>Time</i>	<i>Date</i>	<i>Glycogen, per cent</i>
Right sartorius.....	2:30 p.m.	December 10	0.025
Left sartorius.....	6:05 a.m.	December 12	0.043
Right gracilis.....	2:32 p.m.	December 10	0.027
Left gracilis.....	6:06 a.m.	December 12	0.039
Right adductor.....	2:35 p.m.	December 10	0.065
Left adductor.....	6:09 a.m.	December 12	0.072
Right quadriceps.....	2:33 p.m.	December 10	0.067
Left quadriceps.....	6:09 a.m.	December 12	0.075

Experiment 4. The weight of the dog was 8.9 kgm. The abdominal wound had healed three weeks following pancreatectomy. The animal

was placed in the cold for a period of eight hours, after which the first specimens of muscle were removed under local anesthesia. Subsequently glucose was injected intravenously at the rate of 1.5 grams for each kilogram of body weight per hour for twelve hours. The glucose-nitrogen ratio during the twelve hours prior to beginning the experiment was 2.1:1. The second specimens were secured at the time of death. Estimations of glycogen were as follows:

<i>Muscle</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Left sartorius.....	7:02 p.m.	0.090
Right sartorius.....	6:35 a.m.	0.040
Left quadriceps.....	7:04 p.m.	0.080
Right quadriceps.....	6:37 a.m.	0.060
Left gracilis.....	7:05 p.m.	0.076
Right gracilis.....	6:38 a.m.	0.010
Left adductor.....	7:06 p.m.	0.116
Right adductor.....	6:39 a.m.	0.070

Experiment 5. The weight of the dog was 4.4 kgm. The abdominal wound had healed in a little less than three weeks. The first specimens of muscle and liver were secured at 8:40 to 9:10 a.m. From 9:00 a.m. to 3:00 p.m., 1.0 gram of glucose for each kilogram of body weight per hour was injected intravenously. The second specimens were then removed. The glucose-nitrogen ratio prior to beginning the experiment was 1.2:1. Estimations of glycogen were as follows:

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Liver.....	8:58 a.m.	0.204
Liver.....	3:30 p.m.	0.140
Right sartorius.....	9:06 a.m.	0.030
Right sartorius.....	3:35 p.m.	0.020
Right quadriceps.....	9:08 a.m.	0.030
Right quadriceps.....	3:37 p.m.	0.060
Right gracilis.....	9:09 a.m.	0.020
Right gracilis.....	3:38 p.m.	0.010
Right adductor.....	9:10 a.m.	0.104
Right adductor.....	3:39 p.m.	0.070

Experiment 6. The weight of the dog was 11.4 kgm. The wound had completely healed at the end of three weeks. Specimens of muscle and liver were secured at 8:20 to 9:00 a.m. From 9:00 a.m. until 3:00 p.m.,

1.5 grams of glucose for each kilogram of body weight was injected intravenously. The animal remained in good condition throughout the experiment except that there was considerable shivering of the extremities. The glucose-nitrogen ratio was 3.0:1. Estimations of glycogen were as follows:

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Liver	8:50 a.m.	0.140
Liver.....	3:00 p.m.	1.070
Right sartorius.....	8:55 a.m.	0.255
Right sartorius.....	3:03 p.m.	0.019
Right quadriceps.....	8:56 a.m.	0.185
Right quadriceps.....	3:04 p.m.	0.086
Right gracilis.....	8:57 a.m.	0.455
Right gracilis.....	3:06 p.m.	0.013
Right adductor.....	8:58 a.m.	0.668
Right adductor.....	3:06 p.m.	0.250

Experiment 7. The weight of the dog was 9.4 kgm. The wound healed slowly, and it was not possible to begin the experiment until seven weeks after pancreatectomy. The first specimens of muscle and liver were secured at 8:30 to 9:05 a.m. The final specimens were obtained after the animal had been killed. From 9:30 a.m. to 4:30 p.m. the animal received 2 grams of glucose intravenously for each kilogram of body weight. The glucose-nitrogen ratio during the eighteen hours previous to the injections was 2.56:1. Estimations of glycogen were as follows:

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Liver.....	8:42 a.m.	0.086
Liver.....	4:30 p.m.	0.290
Right sartorius.....	8:53 a.m.	0.072
Left sartorius.....	4:32 p.m.	0.221
Right gracilis.....	8:57 a.m.	0.151
Left gracilis.....	4:34 p.m.	0.238
Right adductor.....	8:58 a.m.	0.050
Left adductor.....	4:36 p.m.	0.326
Right quadriceps.....	8:55 a.m.	0.151
Left quadriceps.....	4.33 p.m.	0.218

Experiment 8. The weight of the dog was 13.4 kgm. Nine weeks after pancreatectomy the abdominal wound had completely healed. The first specimens of liver and muscle were secured at 8:30 to 9:00 a.m. The

final specimens were obtained after the animal had been killed. From 9:00 a.m. to 9:00 p.m. 0.5 gram of glucose for each kilogram of body weight per hour was administered intravenously. The animal remained in good condition throughout the experiment except for marked shivering of the entire body. The glucose-nitrogen ratio during the twelve hours preceding the injection was 2.6:1. Estimations of glycogen were as follows:

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Liver.....	8:45 a.m.	0.528
Liver.....	9:10 p.m.	0.994
Left quadriceps.....	8:50 a.m.	0.024
Right quadriceps.....	9:12 p.m.	0.032
Left adductor.....	8:52 a.m.	0.028
Right adductor.....	9:14 p.m.	0.052

Experiment 9. The weight of the dog was 5.6 kgm. Three weeks after pancreatectomy the abdominal wound had completely healed. The first specimens of muscle and liver were secured at 8:35 to 8:57 a.m. From 9:30 a.m. to 3:00 p.m., 1.0 gram of glucose for each kilogram of body weight was injected intravenously. The glucose-nitrogen ratio during the twenty-four hours previous to beginning the experiment was 3.4:1. Estimations of glycogen were as follows:

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Liver.....	8:42 a.m.	1.00
Liver.....	8:42 a.m.	0.98
Liver.....	3:00 p.m.	1.26
Liver.....	3:00 p.m.	1.28
Right quadriceps.....	8:57 a.m.	0.16
Right quadriceps.....	8:57 a.m.	0.16
Left quadriceps.....	3:02 p.m.	0.06
Left quadriceps.....	3:02 p.m.	0.06
Right adductor.....	8:58 a.m.	0.20
Right adductor.....	8:58 a.m.	0.19
Left adductor.....	3:04 p.m.	0.07
Left adductor.....	3:04 p.m.	0.05

Experiment 10. The weight of the dog was 7.0 kgm. Three weeks after pancreatectomy the abdominal wound had entirely healed. The first specimens of muscle and liver were secured at 8:30 to 9:00 a.m. The second specimens were obtained after the animal had been killed. From 9:00 a.m. to 7:00 p.m., 0.5 gram of glucose for each kilogram of body weight per hour was injected intravenously. The glucose-nitrogen ratio during the twelve hours preceding the experiment was 2.6:1. The animal

shivered excessively during the hour previous to obtaining the final specimens. Estimations of glycogen were as follows:

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Liver.....	8:42 a.m.	0.04
Liver.....	8:42 a.m.	0.05
Liver.....	7:00 p.m.	0.12
Liver.....	7:00 p.m.	0.15
Left adductor.....	8:52 a.m.	0.42
Left adductor.....	8:52 a.m.	0.53
Right adductor.....	7:12 p.m.	0.08
Right adductor.....	7:12 p.m.	0.09
Left quadriceps.....	8:52 a.m.	0.22
Left quadriceps.....	8:52 a.m.	0.20
Right quadriceps.....	8:08 p.m.	0.02

Experiment 11. The weight of the dog was 5.0 kgm. The wound had completely healed three weeks following pancreatectomy. The first specimens of muscle and liver were secured at 12:30 to 1:00 p.m. The final specimens were obtained after the animal had been killed. From 1:00 p.m. to 1:00 a.m. the animal received 0.5 gram of glucose intravenously for each kilogram of body weight per hour. Unlike previous experiments, the dog used in the present study was kept under artificial heat during the course of the experiment to avoid the shivering which had been noted repeatedly in other dogs. The glucose-nitrogen ratio prior to beginning the experiment was 2.9:1. Estimations of glycogen were as follows:

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Liver.....	12:40 p.m.	0.123
Liver.....	12:40 p.m.	0.131
Liver.....	1:05 a.m.	0.563
Liver.....	1:05 a.m.	0.555
Left adductor.....	12:46 p.m.	0.090
Left adductor.....	12:46 p.m.	0.086
Right adductor.....	1:09 a.m.	0.358
Right adductor.....	1:09 a.m.	0.376
Left quadriceps.....	12:50 p.m.	0.075
Left quadriceps.....	12:50 p.m.	0.068
Right quadriceps.....	1:13 a.m.	0.323
Right quadriceps.....	1:13 a.m.	0.342

Experiment 12. The weight of the dog was 7.3 kgm. Three weeks after pancreatectomy the wound had completely healed. The first specimens of liver and muscle were secured at 1:35 to 2:10 p.m. The final specimens were obtained after the animal had been killed. During the experiment the

animal was kept warm and there was no gross shivering. From 2:30 p.m. until 2:00 a.m. the dog received 0.5 gram of glucose intravenously for each kilogram of body weight per hour. The glucose-nitrogen ratio was 3.2:1. Estimations of glycogen were as follows:

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Liver.....	1:43 p.m.	0.136
Liver.....	1:43 p.m.	0.121
Liver.....	2:10 a.m.	0.736
Liver.....	2:10 a.m.	0.718
Left quadriceps.....	1:58 p.m.	0.137
Left quadriceps.....	1:58 p.m.	0.145
Right quadriceps.....	2:17 a.m.	0.421
Right quadriceps.....	2:17 a.m.	0.398
Left adductor.....	2:03 p.m.	0.167
Left adductor.....	2:03 p.m.	0.156
Right adductor.....	2:21 a.m.	0.532
Right adductor.....	2:21 a.m.	0.518

Experiment 13. The weight of the dog was 7.3 kgm. Two weeks after pancreatectomy the abdominal wound had completely healed. The first specimens of liver were secured at 1:30 to 2:00 p.m. The final specimens were obtained after the animal had been killed. Glucose was injected intravenously at the rate of 0.5 gram for each kilogram of body weight from 8:00 p.m. until 4:00 a.m. During the experiment the animal was kept warm. The glucose-nitrogen ratio prior to beginning the experiment was 3.2:1. Estimations of glycogen were as follows:

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Liver.....	7:23 p.m.	0.086
Liver.....	7:23 p.m.	0.078
Liver.....	4:05 a.m.	0.834
Liver.....	4:05 a.m.	0.819
Left adductor.....	7:42 p.m.	0.101
Left adductor.....	7:42 p.m.	0.093
Right adductor.....	4:11 a.m.	0.509
Right adductor.....	4:11 a.m.	0.516
Left quadriceps.....	7:46 p.m.	0.081
Left quadriceps.....	7:46 p.m.	0.091
Right quadriceps.....	4:14 a.m.	0.438
Right quadriceps.....	4:14 a.m.	0.453

Experiment 14. The weight of the dog was 8.8 kgm. Twelve days after pancreatectomy the abdominal wound had almost healed. The first specimens of muscle and liver were secured at 7:20 to 7:50 p.m. From 8:00 p.m. to 3:30 a.m. 0.5 gram of glucose was injected intravenously for

each kilogram of body weight per hour. The animal was kept warm throughout the experiment. The glucose-nitrogen ratio during the twelve hours prior to beginning the experiment was 3.1:1. The animal shivered despite the fact that it was kept warm. Estimations of glycogen were as follows:

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Liver.....	7:15 p.m.	0.201
Liver.....	7:15 p.m.	0.196
Liver.....	3:35 a.m.	0.181
Liver.....	3:35 a.m.	0.169
Right adductor.....	7:31 p.m.	0.087
Right adductor.....	7:31 p.m.	0.077
Left adductor.....	3:42 p.m.	0.023
Left adductor.....	3:42 p.m.	0.017
Right quadriceps.....	7:35 p.m.	0.074
Right quadriceps.....	7:35 p.m.	0.083
Left quadriceps.....	3:45 a.m.	0.042
Left quadriceps.....	3:45 a.m.	0.038

Experiment 15. The weight of the dog was 5.5 kgm. The wound had completely healed four weeks after pancreatectomy. The first specimens of muscle and liver were secured at 6:20 to 6:55 p.m. The second specimens were secured after glucose had been injected intravenously for a period of six hours at the rate of 0.5 gram for each kilogram of body weight per hour. After the second specimens had been obtained, glucose was injected intravenously at the rate of 1.0 gram for each kilogram of body weight per hour, and in addition insulin was injected intravenously at the rate of 5 clinical units per hour. After six hours of such treatment the animal was killed and the final specimens were obtained. The glucose-nitrogen ratio during the twelve hours previous to beginning the experiment was 3.0:1. Estimations of glycogen were as follows:

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Liver.....	6:29 p.m.	0.096
Liver.....	6:29 p.m.	0.087
Liver.....	12:15 a.m.	0.431
Liver.....	12:15 a.m.	0.426
Liver.....	6:10 a.m.	1.236
Liver.....	6:10 a.m.	1.189
Right quadriceps.....	6:43 p.m.	0.019
Right quadriceps.....	6:43 p.m.	0.030
Left quadriceps.....	12:24 a.m.	0.327
Left quadriceps.....	12:24 a.m.	0.311
Right quadriceps.....	6:17 a.m.	0.934
Right quadriceps.....	6:17 a.m.	0.958

<i>Muscle or liver</i>	<i>Time</i>	<i>Glycogen, per cent</i>
Right adductor.....	6:47 p.m.	0.231
Right adductor.....	6:47 p.m.	0.241
Left adductor.....	12:28 a.m.	0.398
Left adductor.....	12:28 a.m.	0.415
Right adductor.....	6:20 a.m.	1.126
Right adductor.....	6:20 a.m.	1.200

COMMENT. It may be noted that the results of the foregoing experiments are not uniform. In the first experiments two factors were not adequately controlled: 1, too large doses of glucose were administered which produced an unfavorable general reaction in the animal, and 2, in the earlier experiments shivering was not controlled. By keeping the dogs warm shivering was either obliterated or minimized.

It was found that during administration of glucose, glycogen in the liver decreased in five animals; it was slightly, although not significantly increased in one animal, and it was appreciably augmented in nine animals. The glycogen in muscles was decreased in eight animals; it was slightly, but not significantly increased in one animal, and it was definitely increased in five animals.

These results are interesting because the positive data are much more convincing than the negative. In none of the reported experiments was pancreatic tissue found after careful search at necropsy, and the animals appeared to be in a complete state of diabetes.

It appears from these experiments that the depancreatized organism is capable of forming glycogen in both liver and muscle, but it is not formed in amounts comparable with that of the normal animal or of the diabetic animal under treatment with insulin. The question of oxidation of this stored carbohydrate is beyond the scope of this paper.

SUMMARY

The depancreatized dog, five days after withdrawal of insulin, is capable of forming glycogen in both skeletal muscle and liver, although at a rate far below that of a normal dog.

BIBLIOGRAPHY

- (1) BEST, C. H., J. P. HOET AND H. P. MARKS. *Proc. Roy. Soc. London*, s.B, 1926, c, 32.
- (2) BEST, C. H., C. M. JEPHCOTT AND D. A. SCOTT. *This Journal*, 1932, c, 285.
- (3) BODO, R. C., F. C. TUI AND L. A. FARBER. *This Journal*, 1932, ci, 10.
- (4) CHAIKOFF, I. L. *Journ. Biol. Chem.*, 1927, lxxiv, 203.
- (5) CHOI, Y. O. *This Journal*, 1928, lxxxiii, 406.
- (6) CRUICKSHANK, E. W. H. *Journ. Physiol.*, 1913, xlvii, 1.
- (7) FISHER, N. F. AND R. W. LACKEY. *This Journal*, 1925, lxxii, 43.
- (8) FRASER AND MACLEOD: Quoted by MACLEOD (11).

- (9) KERMACK, W. O., C. G. LAMBIE AND R. H. SLATER. *Biochem. Journ.*, 1929, xxiii, 416.
- (10) MACLEOD, J. J. R. *The fuel of life*. Princeton University Press, 1928, 147 pp.
- (11) MACLEOD, J. J. R. *Lancet*, 1929, ii, 1.
- (12) MACLEOD, J. J. R. AND J. MARKOWITZ. *Trans. Assoc. Amer. Physic.*, 1926, xli, 147.
- (13) MARKOWITZ, J., F. C. MANN AND J. L. BOLLMAN. *This Journal*, 1929, lxxxvii, 566.
- (14) SOSKIN, S. *Journ. Nutrition*, 1930, iii, 99.

THE ANTIRACHITIC EFFICIENCY OF NEW ORLEANS SUNSHINE¹

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The chief factor in the seasonal incidence of rickets is the seasonal variation of the sun's altitude. This determines the quality and quantity of ultra violet, particularly that shorter than $313\text{ m}\mu$. The actual number of sunshine hours is of relatively little significance, as illustrated, for instance, by the conditions in the Panama Canal Zone, where rickets is practically non-existent, but where the yearly sunshine is less than in New York City, and less evenly distributed (Hess, 1925).

New Orleans, a sub-tropical city, is of particular interest in this connection. Although the mean temperature of New Orleans is high, the Weather Bureau records show an average for the last forty years of only 58 per cent sunshine out of the total possible number of hours, an amount not greater than that in many other cities in this country. Furthermore, being practically at sea level and having a very humid climate (average relative humidity = 76 per cent), it might be expected that the antirachitic wave lengths would be absorbed and their intensity thus considerably diminished in passing through the atmosphere. The mild temperature, however, allows infants and children to be out of doors throughout most of the year. The availability of green vegetables, ample playing space, and light clothing all tend to minimize the incidence of rickets. Williams (1928) and Crawford and Williamson (1930) report that although mild rickets is quite prevalent amongst infants in New Orleans, severe rickets is seldom encountered.

In none of the local studies has there been any attempt to correlate the incidence and severity of rickets with the variations in the antirachitic wave lengths of sunlight, a factor which, in view of our present knowledge, assumes the utmost importance. We have determined the minimum effective antirachitic exposures during the different seasons of the year using rats and chicks, and have correlated these biological data with physical measurements of the solar antirachitic radiation present at the time of

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exposure of the animals. In addition to exposing the animals to direct sunshine and to skyshine (reflected radiation from sky and clouds), we have also exposed parallel groups under Vitaglass, Corex D and Cel-O-Glass.²

Rats bred from our stock colony and weighing about 40 grams were divided into groups of 5 or 6 and fed the Steenbock no. 2965 rachitic diet, with distilled water *ad libitum*. Each experiment included a control group in comparison with which the diagnosis was made, for the most part on the basis of the line test and roentgenograms of the femurs and tibias. In some of the early experiments, serum phosphorus determinations were also made. The duration of almost all of the experiments was 5 weeks which is usually sufficient for definite rickets to develop in control groups kept in the animal room, and in only a few instances was it necessary to continue the experiments longer.

In a parallel study, two day old white leghorn chicks of uniform stock were placed in a brooder and fed a diet composed of 57 parts yellow corn, 20 parts powdered skim milk, 20 parts wheat middlings, $\frac{1}{2}$ part salt and $2\frac{1}{2}$ parts ground bone meal. Tap water was given *ad libitum*. When two weeks old, the chicks were weighed, banded and divided into groups of seven to ten each and placed in large cages. Each experiment included two control groups. Two per cent cod liver oil was added to the diet of the first of these and this group was considered as normal. No addition was made to the diet of the second and these birds served as the leg weakness controls. The chicks were weighed weekly and when 8 weeks old were x-rayed and sacrificed, the blood being pooled for serum calcium and phosphorus determinations. Our estimates as to the efficiency of the radiation were based upon a comparison of the general appearance, the growth curves, roentgenograms and calcium and phosphorus analyses with those of the two control groups.

The rats and chicks were kept in adjoining rooms, which were well ventilated, dimly illuminated and of fairly constant temperature. Certain groups of animals were exposed to sunshine and skyshine on the roof of the animal building adjoining the laboratory which is situated on the college campus, about 4 miles away from the business center of the city, at a latitude of $29^{\circ} 56' 2''$ and about 35 feet above sea level. The atmosphere is reasonably clear of smoke and dust, although the animals were cut off from some of the radiation from the sky by high trees and buildings. The groups exposed to skyshine were placed on the same roof but in the shadow of the building, receiving radiation from all directions except south. The exposures were begun at 10 a.m. and made daily except on extremely cloudy or rainy days. Consequently in many cases an exposure to sun-

² These various screens were kindly presented to us by the manufacturers, Vitaglass Corporation, Corning Glass Works and Acetol Products, Inc., respectively, to whom we here express again our thanks.

shine means that sunshine was available for a definite percentage of this time, while for the remainder, the animals received skyshine or reflected energy from sky and clouds. Several pieces of each of the screens used (Vitaglass, Corex D and Cel-O-Glass) were measured for cut-off and transmission before being exposed and at intervals later. They showed no effect of solarization after 15 months of use, undoubtedly due to the fact that the total time for which they were exposed was relatively short.

The measurement of the solar and sky radiation was done on a tower built on the roof of the animal house adjacent to where the rats were being exposed. Such measurements were begun in 1928 and are still being continued. The extensive information we have thus obtained as to the variation in the total energy (intensity) and the distribution (quality) of sunshine and skyshine in New Orleans will be reported in detail elsewhere, only the pertinent data being referred to here. Spectrograms of the sun were made with a Zeiss spectrograph. The intensity of the total energy of direct sunlight was determined by means of the pyrhelimeter described by Gorczynski (1924), the recording millivoltmeter of which was supplemented by a high sensitivity Leeds and Northrup galvanometer. To obtain the distribution of the energy, screens with various cut-offs were used. The intensity of the antirachitic radiation was measured by interposing in front of the thermopile a piece of window glass with a transmission of 0.5 per cent at $313\text{ m}\mu$, 49 to 50 per cent at $334\text{ m}\mu$, 85 per cent at $365\text{ m}\mu$ and about 90 per cent in the visible and near infra red. The long wave limit of the ultra violet cut-off is taken at (and including) $313\text{ m}\mu$ (instead of $310\text{ m}\mu$, the absolute cut-off) because it is a definite and easily obtained emission line (of the mercury arc spectrum) and, also, because antirachitic action terminates practically at this wave length.

Exposure to sunshine. A total of thirty-three experiments was made on rats, extending from May 31, 1929 to December 26, 1931, the results of which are shown in table 1. As mentioned above, when animals are exposed to sunshine they also receive a large amount of radiant energy from the hemispherical dome of the sky; when it is cloudy the ratio of this skyshine to direct sunshine is high. We have indicated the total and average times of exposure to sunshine (sun + sky, columns 4 and 6) and to skyshine alone, that is to say, when the sun was obscured by clouds (sky, columns 5 and 7). During particularly cloudy months, the rats actually received more skyshine than sunshine, as in experiments 2, 19, 20, 21, 27, 28 and 30. The discrepancies in the sums of the average values (columns 6 and 7) and the daily exposures (column 3) are due to the variation in the number of rainy or very cloudy days.

A daily exposure of from 10 to 30 minutes (average 2.7 to 14.0 minutes) to sunshine and skyshine was more than adequately protective in the first eight experiments, extending from May 31, 1929, through February 14,

TABLE 1
Exposure of rats to sunshine and skyshine

EXPERIMENT NUMBER	DURATION	DAILY EXPOSURE	TOTAL TIME				AVERAGE DAILY EXPOSURE		AVER- AGE ENERGY VALUE, DAILY EX- POSURE	DIAGNOSIS
			Sun + Sky		Sky		Sun + Sky	Sky		
	1929	min- utes	hours	min- utes	hours	min- utes	min- utes	min- utes	gm. cal./cm. ²	
1	May 31-July 6	10	1	40	1	10	2.7	2.0	0.00119	—
2	July 24-Aug. 29	30	5	35	9		9.5	15.0	0.00548	—
3	Sept. 26-Oct. 30	30	9	15	4	45	16.0	8.1	0.00614	—
4	Oct. 6-Nov. 12	10	3	5	1	50	5.1	3.0	0.00270	—
5	Oct. 30-Dec. 4	30	7		5		11.7	8.3	0.00423	—
6a	Nov. 16-Dec. 20	10	3	10	1		5.4	1.7	0.00179	—
6b	Nov. 16-Dec. 20	20	6	20	2		10.8	3.4	0.00358	—
7	Dec. 5-Jan. 17	30	8		6		14.0	10.0	0.00195	—
	1930									
8	Jan. 3-Feb. 14	10	3	10	1	40	4.5	2.4	0.00102	—
9	Feb. 10-Mar. 24	10	2	20	1	20	3.3	1.9	0.00071	4-; 2++
10	Feb. 24-Mar. 31	5		45		30	1.3	0.8	0.00045	3+++; 3+++++
12	Mar. 29-May 1	10	3		1	20	5.5	2.4	0.00290	—
14	May 3-June 7	5	1	15		50	2.2	1.4	0.00167	—
15	May 17-June 24	5	1	45		50	2.7	0.8	0.00158	—
17a	June 16-July 21	5	2	10		20	3.7	0.6	0.00217	—
17b	June 16-July 21	5*	1			15	1.7	0.1	0.00099	3-; 2++
19a	July 25-Sept. 4	8	1	20	2	16	1.9	3.3	0.00086	—
19b	July 25-Sept. 4	10	1		1	40	1.4	2.4	0.00064	—
20	Sept. 5-Oct. 10	5	1	5	1	20	1.9	2.1	0.00052	—
21	Nov. 24-Dec. 29	20	3	20	4		5.4	6.7	0.00175	—
	1931									
22	Jan. 5-Feb. 9	10	2	30	1		4.3	1.7	0.00086	2+++; 2++++
23	Feb. 19-Mar. 26	15	4	40	1	30	8.0	2.7	0.00308	—
24	Apr. 7-May 12	5	1	20	1		2.3	1.7	0.00096	—
26	May 16-June 20	5	1	30		50	2.6	1.4	0.00115	—
27	June 5-July 11	5	1	5	1	20	1.8	2.2	0.00061	—
28	June 24-July 29	5		55	1	10	1.6	2.0	0.00054	—
29a	July 17-Aug. 20	5*	1			15	1.7	0.4		—
29b	July 17-Aug. 20	5†		40		15	1.1	0.4		—
30	July 31-Sept. 1	5		50	1	15	1.6	2.3		—
31a	Aug. 24-Sept. 26	5	1	10	1	10	2.1	2.1		—
31b	Aug. 24-Sept. 26	5†		30			0.9	0.0		—
32	Sept. 30-Nov. 4	5	1	25		50	2.4	1.4	0.00090	2-; 3++
33	Nov. 21-Dec. 26	8	1	44		24	2.7	0.7		1-; 5+

* = 3 times each week; † = twice a week; ‡ = on clear days only.

— = no rickets; + = borderline; ++ = mild; +++ = moderate; ++++ = florid rickets.

1930. Since our chief aim was to determine the seasonal variation in the minimum amount of antirachitic radiation in New Orleans sunlight, we accordingly shortened the exposures in the succeeding experiments. The minimum individual exposure time was set at five minutes, and in some cases was obviously longer than necessary. To secure further information on this point, a few groups of rats, while exposed for 5 minutes, were put out only 2 or 3 times a week. An average daily exposure to sunshine (and skyshine) of between 2 and 3 minutes was amply antirachitic from April through October. Of particular interest are experiments 1, 15 and 26 during similar periods (May and June) of 1929, 1930, and 1931, in which protection was afforded by average daily exposures of 2.7, 2.7 and 2.6 minutes, respectively. The minimum effective exposure from November through March was found to be from 5 to 6 minutes, shorter exposures during this period (expts. 9, 10, 22 and 33) being only partially protective. We have included experiments 9, 10 and 23, performed during the latter half of February and March, in this grouping, although the minimum effective time used was 8 minutes (expt. 23). Since 3.3 minutes (expt. 9), however, was almost completely protective, we have taken the mean value as representing the approximate threshold. Note should be made that although an average daily exposure of 1.3 minutes during the latter part of February and March (expt. 10) was followed by severe rickets, an average daily duration of 1.4 minutes was protective from July 25 to September 4 (expt. 19b).

Vitaglass was first used in experiment 7 and in fifteen subsequent experiments, Corex D in eight and Cel-O-Glass in seven experiments. The requisite increase in the duration of the exposures when these screens are used is proportional to the absorption of antirachitic radiation by the screens. For example, with Vitaglass the effective calculated exposure is from 2 to 2.5 times as long as to direct sunlight. The calculated effective exposures through Vitaglass are from 4 to 7.5 minutes for April to October, inclusive, and from 10 to 15 minutes from November to March, inclusive. For Corex D, the values are from 3 to 4 minutes and from 6 to 8 minutes, and for Cel-O-Glass from 7 to 10 and from 17 to 20 minutes, respectively. Our actual findings are in general agreement with these calculated values, thus demonstrating that our average exposures to direct sunshine (without the screens) were fairly close to the minimum durations necessary for the prevention of rickets.

The experiments on chicks were started on October 16, 1929, and terminated on January 2, 1932. The data are given in table 2. They show that average daily exposures of about 2 minutes to sunshine and skyshine during July, August and September; of between 2 and 3 minutes during April, May, June and October; of 4 to 5 minutes during November, December and January; and of 3 to 5 minutes during February and March, will pre-

TABLE 2
Exposure of chicks to sunshine and skyshine

EXPERIMENT NUMBER	DURATION	DAILY EXPOSURE	TOTAL TIME				AVERAGE DAILY EXPOSURE		AVERAGE ENERGY VALUE DAILY EXPOSURE gm. cal./cm. ²	AVERAGE WEIGHT AT 8 WEEKS grams	Ca mgm. per 100 cc.	P mgm. per 100 cc.	DIAONOSIS
			Sun + Sky		Sky		Sun + Sky	Sky					
			hours	min-utes	hours	min-utes							
	1929												
2	Oct. 16-Nov. 27	30	11	30	3		16.0	4.3	0.00397	331	11.10	6.04	—
3	Nov. 6-Dec. 16	10	2	50	2		4.2	3.0	0.00073	302	12.72	5.56	—
4	Dec. 5-Jan. 10	30	6	30	7		11.0	11.6	0.00270	381	9.76	5.00	—
	1930												
5	Jan. 4-Feb. 14	10.	3	10	1	30	4.6	2.2	0.00108	331	13.9	6.08	—
6	Feb. 18-Mar. 31	10	2	55	1		4.2	1.4	0.00162	328	11.5	4.85	—
7	Mar. 20-May 9	5	1	50	1	20	2.2	1.6	0.00113	424	13.4	6.12	—
8	Apr. 5-May 23	10	3	50	2	10	4.8	2.7	0.00226	500	13.4	5.64	—
11	Aug. 5-Oct. 1	5	1	50	1	30	1.9	1.6	0.00076	444	13.9	5.28	—
12	Oct. 6-Nov. 15	5	1	30		35	2.3	0.9	0.00069	423	12.7	5.64	—
13	Nov. 18-Dec. 30	5		50	1		1.2	1.4	0.00026	291	11.7	2.84	3-; 3++
	1931												
14	Dec. 31-Feb. 9	7.5	1	30	1	20	2.1	2.0	0.00046	259			2+; 3+++; 3++++
													—
15	Feb. 19-Apr. 1	8	2	30		32	3.6	0.8	0.00142	338			—
16	Apr. 7-May 16	5	1	45	1		2.6	1.5	0.00112	350	13.37	5.64	—
17	May 19-June 27	5	1	45		50	2.6	1.2	0.00141	376	13.08	5.44	—
18	July 2-Aug. 13	5	1	25	1	15	2.0	1.8	0.00104	512		5.46	—
19	Sept. 16-Oct. 27	5*	1	10	1		2.4	1.7	0.00090	366	13.5	5.70	—
20	Nov. 18-Jan. 2	8	2	32		24	3.6	0.5		310		4.52	—

* = 4 times a week.

— = no, + = mild, ++ = moderate, +++ = severe leg weakness.

vent the appearance of leg weakness and promote normal growth. Average daily exposures of from 2.0 to 3.8 minutes through Vitaglass or Corex D prevented leg weakness and stimulated normal growth from April through October, and 4.5 to 5.4 minutes was adequate during the rest of the year. The fact that in several experiments the same average daily exposures to sunshine with and without the screens were effective unquestionably indicates that the exposures without the screens were well above the minimum and that shorter average daily exposures would have been ample. The results with Cel-O-Glass show that, even with its relatively poor transmission (27 to 30 per cent), average exposures of from 5 to 6 minutes are adequate in May and June and in September and October, and partially so in January.

TABLE 3
Average amounts of antirachitic radiation during the year at 10 a.m.

DATE	NUMBER OF DAYS MEAS- URED	GM. CAL. PER SQ. CM. PER MINUTE	μ W PER SQ. CM.	NUMBER OF DAYS MEAS- URED	GM. CAL. PER SQ. CM. PER MINUTE	μ W PER SQ. CM.
	1930			1931		
Jan.....	3	0.000252	17.64	6	0.000226	15.82
Feb.....	5	0.000213	14.91	5	0.000195	13.65
Mar.....	5	0.000385	26.95	5	0.000401	28.07
Apr.....	6	0.000528	36.96	3	0.000485	33.95
May.....	9	0.000563	39.41	4	0.000453	31.71
June.....	4	0.000836	58.52	4	0.000565	39.55
July.....				2	0.000634	44.38
Aug.....	3	0.000572	40.04	1	0.000737	51.59
Sept.....	3	0.000452	31.64			
Oct.....	7	0.000373	26.11	4	0.000374	26.18
Nov.....	6	0.000278	19.46	3	0.000274	19.18
Dec.....	5	0.000183	12.81			

During the winter months there is very little radiant energy shorter than $313\text{ m}\mu$ in New Orleans sunlight except on brilliantly clear days. The average values for the months of the year and the number of determinations from which they were derived are given in table 3. It is to be remembered that these averages were obtained from measurements made on clear days only or on days when the sun was not obscured by clouds. Since there are more cloudy and rainy days than there are clear days in New Orleans (average for 60 years shows only 124 clear days annually), the values given represent the approximate upper limits that may be expected. Coblenz, Stair and Hogue (1931) obtained values ranging from 60 to $80\text{ }\mu\text{w}$ (microwatts) at 10 a.m. during June in Washington and about $57\text{ }\mu\text{w}$ in July. They conclude that the average amount of ultra violet

radiation of wave lengths less than and including $313\text{ m}\mu$ for mid-latitude sea level stations, during the clearest weather, during midday in midsummer, amounts to about $0.00129\text{ gm. cal. per sq. cm. per min.}$ ($90\text{ }\mu\text{w}$ per sq. cm.) decreasing to about $0.000285\text{ gm. cal. per sq. cm. per min.}$ ($20\text{ }\mu\text{w}$ per sq. cm.) on the clearest days in midwinter. Our midsummer, midday values are of the same order, ranging from 65 to $70\text{ }\mu\text{w}$, although we sometimes obtained values of 38 to $49\text{ }\mu\text{w}$ on clear days in midwinter. The weather for the year 1930 was considered by the local Weather Bureau as remarkably near the normal, or long term mean, and the total number of determinations represents over one-third of the clear days during the year. The differences in the values for similar months in 1930 and 1931 reflect the variations in the amount of haze and clouds present at the time. The single value for August, 1931, was obtained on an unusually clear, dry day and is probably higher than an average of several days would have been, since the weather in New Orleans during July and August is usually very cloudy, the average number of clear days for the last 56 years being only 7 and 8, respectively, as compared with 10 in December and 16 in October. DeBuys (1924) and DeBuys and von Meysenbug (1924) found that the peak of incidence of rickets in infants in New Orleans varied in different years. Thus, in 1922 they found the greatest incidence during March, while in the following year, the peak had shifted to late in July. Our findings for 1930 and 1931 clearly demonstrate that the reason for this variation in time of incidence is the variation in the amount of radiation shorter than $313\text{ m}\mu$.

Spectrograms, taken while the animals were being exposed (9:00–10:30 a.m.), show that at this time of the day the spectrum very rarely extends to $300\text{ m}\mu$ and we have only one record on June 6, 1930, of $299\text{ m}\mu$. During the winter months the spectrum seldom extends below $306\text{ m}\mu$ except on very clear days after a rain storm when the air has been washed clean. Throughout the year, in fact, there are relatively few days on which between 11 a.m. and 1 p.m. the spectrum extends below $304\text{ m}\mu$. From June 1 to December 31, 1929, only 5 out of 55 days measured showed limits shorter than $304\text{ m}\mu$, only 7 days out of 77 in 1930, and only 15 days out of 50 in 1931. Air pollution, clouds and haze decrease the extent of the spectrum so that even in August for days on end there are no wave lengths shorter than $310\text{ m}\mu$. In many cases there must be considerable carry-over of antirachitic effect from those days when the shorter wave lengths are present (see Russell and Howard, 1931). In experiment 31b (table 1), six 5 minute exposures on clear days over a period of 5 weeks protected a group of rats as adequately as did a 5 minute daily exposure of another group for the same length of time. Since solar radiation of the most effective antirachitic wave lengths, $302\text{ m}\mu$ and shorter, is so seldom present in this city, and since it is possible at all times of the year to prevent

rickets in rats and chicks by exposing them to the available sunshine, the conclusion is obvious that the effects are being exerted, for the most part, by wave lengths between 304 and 313 $m\mu$.

The smallest amount of solar energy of wave lengths less than 313 $m\mu$ protective for the rats (see table 1) was an average of 0.00052 gm. cal. per sq. cm. daily (expt. 20), a total of 0.0182 gm. cal. per sq. cm. The usual shortest wave length present in the sunshine during this period was 304 $m\mu$ or longer, the spectrum extending to 301 $m\mu$ on one of the days measured. Such values do not represent all of the radiation which the animals received. The pyrliometer with which they were obtained measures only the direct solar radiation, giving no information as to the energy in the diffusely reflected radiation from the sky and clouds which the animals also receive. Our results show that an average daily exposure of rats to somewhat less than 0.001 gm. cal. per sq. cm. of wave lengths shorter than 313 $m\mu$ was partially or completely antirachitic from April through October, while exposures of from 0.001 to 0.002 gm. cal. per sq. cm. were effective during the remainder of the year. The minimum amount of energy effective in preventing leg weakness and in promoting growth in the chicks was 0.00069 gm. cal. per sq. cm. daily, or a total of 0.0290 gm. cal. per sq. cm. The next smallest amount of energy which was completely adequate for calcification and growth was 0.0378 gm. cal. per sq. cm. and in every case where amounts greater than this were used there was normal development of the chicks.

The effective exposures to sunshine which we used are shorter than any that have been published. Day (1932) reported from Little Rock, Arkansas, that the average daily amount of sunshine necessary to afford the same partial protection against rickets in rats receiving a rachitogenic diet varied from 5 minutes in May, June and July to 168 minutes in December, with intermediate values for the other months. Stein and Lewis (1931) at Denver, during June and July, 1927, found an average daily exposure to sunshine of 1.957 minutes inadequate, of 3.75 minutes partially and longer than 7.5 minutes more or less completely protective. Lewis, Frumess and Stein (1931) failed to find a seasonal variation in antirachitic efficiency in Denver because, as they claim, a certain minimum amount of the antirachitic wave lengths reaches the earth throughout the year, owing to the high percentage of sunshine, the low humidity and the small amount of smoke in the atmosphere. Godfrey (1931) also reported the lack of any seasonal variation in solar antirachitic efficiency in Honolulu. Sloan (1929), in a study of the seasonal variation of the solar antirachitic radiation in Ithaca, N. Y. (latitude $42^{\circ} 30' N$, 283 M) found that 30 to 40 minute daily exposures of chicks under Corex A at high noon were about threshold for the prevention of leg weakness during the winter, 10 minutes in the spring, and 2.5 to 5 minutes not quite adequate in the

summer. There is still considerable difference between his findings and ours, especially during the winter months, even after reducing his values by 10 to 15 per cent to correct for the transmission loss through Corex A.

The relatively high intensity of the available radiation in New Orleans throughout the year undoubtedly accounts for the short exposures necessary to prevent rickets. Tisdall and Brown (1929, 1931) report that there is a marked increase in the antirachitic effect of sunshine in Toronto when the sun reaches a midday altitude of 35° late in February and a marked decrease at about the same altitude in October. The increased antirachitic effect with the sun at 35° is due to a general increase in the intensity of the rays ranging from 320 to 294 $m\mu$ rather than to the presence of shorter rays which do not get through with the sun at the lower altitudes. Dick (1922) showed that severe rickets was rare, or absent, in all places where the minimal seasonal altitude of the sun was above 35° , and Tisdall and Brown believe that severe rickets occurs in those places where the altitude of the sun is below 35° for several months of the year. The minimal seasonal midday altitude of New Orleans is 37° so that one would expect sufficient antirachitic radiation present at all times of the year. Our findings accord with this supposition and show that the seasonal variation which we observed was in great measure due to the fact that the solar altitude in New Orleans during November, December and January at 10 a.m. (at which time the animals were exposed) is 32° , 29° and 32° , respectively, and above 35° from February through October. Tisdall and Brown (1931) believe that the reason why Lewis, Frumess and Stein did not find a seasonal variation in solar antirachitic radiation was probably due to the fact that their experiments were done in February, March and April, at which time the sun in Denver has already reached an altitude of 35° and therefore has a high antirachitic potency. Taking into account the latitude and altitude (1650 M) of Denver, Tisdall and Brown would expect to find in that city a low antirachitic effect of sunshine only from December 1 to January 15. The difference between the latitude of Ithaca ($42^\circ 30' N$) and of New Orleans ($29^\circ 56' N$) accounts in some measure for the difference in the duration of minimal effective exposures in Sloan's study and in ours, since the minimal seasonal midday altitude of the sun in Ithaca is only 24° , rising to about 37° in February and to a maximum of 71° in June. The midday altitude of the sun in New Orleans at this time is about 83° .

Exposure to skyshine. The term *skyshine* is used to designate the sun's rays that are reflected from the sky and clouds, in contradistinction to the rays received from the sun itself. It is obvious that animals or infants exposed to sunshine also receive skyshine. The importance of skyshine as a source of ultra violet radiation was first pointed out by Dorno (1919). He showed that at Davos (altitude 1560 M), even under the best conditions and with a solar altitude of 60° , the ultra violet radiation of wave lengths

less than 366 $m\mu$ in direct sunshine was only 90 per cent of that in skyshine. With lower solar altitudes the ultra violet in skyshine increases markedly, so that with a solar altitude of 30° , sunshine contains only about 30 per cent as much ultra violet as skyshine. Our own measurements during the month of July, 1931, show that in New Orleans at 10 a.m., with a solar altitude of about 61° , the intensity of the ultra violet of wave lengths less than 313 $m\mu$ is about 20 per cent greater in skyshine than in direct sunshine.

Our experiments were designed to determine the minimal exposures to skyshine throughout the year which would prevent rickets and to compare these results with those obtained on exposure to sunshine and skyshine. The methods have been outlined above. The same screens were used as in the sunshine experiments. The results of exposure of rats to skyshine are given in table 4. An average daily exposure to skyshine of from 16 to 17 minutes is close to the minimum effective amount which will prevent rickets in rats in New Orleans from May through September, although somewhat shorter exposures during June, July and August, 1931, were protective. Thus the antirachitic action of skyshine is at least one-sixth as potent as that of sunshine, including skyshine, during these months.

Average daily exposures to skyshine of from 22 to 24 minutes (about 4 times those of sunshine + skyshine) were adequate during October, November and December. The exposures of 67 and 44.6 minutes used in January (expts. 8 and 22) were unquestionably too long. Judging from the average exposures found adequate in December and February, an average daily exposure of from 22 to 28 minutes would have been effective. The results for February and March, 1930 and 1931, vary. Thus an average daily exposure of 28 minutes from February 10 to March 24, 1930 (expt. 9) was not quite sufficient, although an average daily exposure of 16 minutes from February 19 to March 26, 1931 (expt. 23) was protective. Similar durations of exposure (15 and 16 minutes) during March of 1930 and 1931 (expts. 10 and 23) resulted in florid rickets in the former, and no rickets in the latter case. The effective exposures of 47 minutes (expt. 12) and 25 minutes (expt. 24) during April were probably longer than necessary. We may conclude that an average daily exposure of somewhat less than 28 minutes would be effective in February and that exposures of from 16 to 20 minutes are protective in March and April.

Partial or complete protection was afforded to rats exposed daily to skyshine under Vitaglass from May through September for average periods of from 28.8 to 50 minutes. This was to have been expected from the transmission data of this screen. Similarly from October through January average daily exposures of from 51.1 to 67 minutes were antirachitic. The results for February, March and April for 1930 and 1931 differ, as did the results on direct skyshine, although the results with and without the screen

are consistent for each year. Exposures of from 28.3 to 50 minutes were adequate in 1931, but not so in 1930. This would indicate that the differences obtained were due to variations in the amounts of antirachitic radiation present at these times in the two years.

TABLE 4
Exposure of rats to skyshine

EXPERIMENT NUMBER	DURATION OF EXPERIMENT	DAILY EXPOSURE	TOTAL TIME		AVERAGE DAILY EXPOSURE	DIAGNOSIS
	1929	minutes	hours	min- utes	minutes	
1	May 31-July 6	10	4	50	8.3	++
2a	June 24-July 29	30	14	5	24.0	-
2b	June 24-July 29	60	28	10	48.0	-
3	Sept. 26-Oct. 30	180	81	35	140.0	-
4	Oct. 8-Nov. 12	30	14	20	24.0	-
5	Oct. 30-Dec. 4	180	72		120.0	-
6a	Nov. 16-Dec. 20	30	13		22.0	-
6b	Nov. 16-Dec. 20	90	36	30	63.0	-
7	Dec. 5-Jan. 17	180	84		148.0	-
	1930					
8	Jan. 3-Feb. 14	90	47	50	67.0	-
9	Feb. 10-Mar. 24	60	20		28.0	4-; 2+
10	Feb. 24-Mar. 31	30	8	30	15.0	++++
12	Mar. 29-May 1	60	26		47.0	-
14	May 3-June 7	30	12	30	22.0	-
15	May 17-June 24	20	9		14.0	1-; 2+++; 2++++
17	June 16-July 21	20	9	40	16.0	1-; 2+++; 2++++
19	July 25-Sept. 4	20	9	20	13.6	++
21	Nov. 24-Dec. 30	60	23		35.0	-
	1931					
22	Jan. 5-Feb. 9	60	26		44.6	-
23	Feb. 19-Mar. 26	45	11		16.0	-
24	April 7-May 12	30	14	30	25.0	-
26	May 16-June 20	20	9	20	16.0	-
27	June 5-July 11	15	7	15	11.8	-
28	June 24-July 29	20	9		15.3	-
29	July 17-Aug. 20	15	7	15	12.4	-
30	July 31-Sept. 1	20	8	20	15.5	-
31	Aug. 24-Sept. 26	20	9	20	16.5	-
32	Sept. 30-Nov. 4	20	9		15.4	3+++; 2++++
33	Nov. 21-Dec. 26	30	8		13.3	3+; 2+++; 1++++

Rats exposed to skyshine under Corex D from June through September obtained almost complete protection with average daily exposures of from 23 to 28 minutes, and average exposures of from 40 to 50 minutes were protective from January through April. The results with Cel-O-Glass show that sufficient protection was afforded during June and July with

average daily exposures of from 23 to 48 minutes, but that relatively long exposures (up to 94 minutes) were inadequate at other times of the year.

The data on exposure of chicks to skyshine are given in table 5. An average daily exposure of between 6 and 11.4 minutes from July through October; of 35 minutes from November through February; and of 18 to 19 minutes from March through June is ample for proper calcification and growth. The latter two values are the means of the actual amounts used

TABLE 5
Exposure of chicks to skyshine

EX- PERI- MENT NUM- BER	DURATION	DAILY EX- POSURE	TOTAL TIME		AVER- AGE DAILY EX- POSURE	AVER- AGE WEIGHT AT 8 WEEKS	Cn	P	DIAGNOSIS
		minutes	hours	min- utes	minutes	grams	mgm. per 100 cc.	mgm. per 100 cc.	
	1929								
2	Oct. 16-Nov. 27	180	89	40	127	342	12.86	5.44	—
3	Nov. 6-Dec. 16	90	42	30	64	345	13.60	5.16	—
4	Dec. 5-Jan. 10	180	69		113	410	10.72	5.48	—
	1930								
5	Jan. 4-Feb. 14	90	42		61	330		5.72	—
6	Feb. 18-Mar. 31	60	19		28	329	10.50	4.92	—
7	Mar. 20-May 9	30	19		23	369	13.40	5.84	—
8	Apr. 5-May 23	60	36		45	361	12.30	4.95	—
11	Aug. 5-Oct. 1	30	19		20	511	11.87	5.88	—
12	Oct. 6-Nov. 15	15	4		6	443	12.0	5.75	—
13	Nov. 18-Dec. 30	10	4		5.9	253		3.30	3++; 4+++
	1931								
14	Dec. 31-Feb. 9	12.5	4	55	7	180			+++
15	Feb. 19-Apr. 1	15	6	45	9.6	259			5-; 1++
16	Apr. 7-May 16	15	8	15	12.4	315	12.31	3.48	1-; 4++; 2+++
17	May 19-June 27	20	10	40	15.6	372	13.32	5.08	5-; 2++
18	July 2-Aug. 13	15	6		11.4	468		5.16	—
19	Sept. 16-Oct. 27	10	5	30	9.4	380	10.1	6.10	—
20	Nov. 18-Jan. 2	20	7	20	10.5	278		4.72	3-; 3++

and represent an approximation of the effective average exposures. Skyshine was thus about one-fourth to one-fifth as effective as was sunshine and skyshine from July through October, and about one-sixth to one-seventh as effective during the rest of the year. Chicks exposed to skyshine under Vitaglass and Corex D require an average exposure of from 11.4 to 14.0 minutes from July through October, from 50 to 60 minutes from November through February, and from 25 to 30 minutes from March through June, for the prevention of leg weakness and stimulation of growth.

The experiments with Cel-O-Glass are of interest because of its extensive use by poultrymen. Complete protection and good growth were obtained in September and October (expt. 19) with an average exposure to skyshine of 28.3 minutes and with 39 minutes during May and June (expt. 17), and even in January, an average daily exposure of 20.3 minutes was partially protective.

In the discussion of the energy equivalents of the sunshine exposures, emphasis was placed on the fact that the values given represented only the amount of direct solar radiation and did not include the diffuse energy received from sky and clouds. During the months of June and July, 1931, we used an Eppley pyrheliometer, the sensitive area of which is exposed, not only to direct solar radiation, but also to that diffusely reflected from the sky and clouds. This instrument, a modification of that designed by Kimball and Hobbs (1923), is thus exposed to radiation similar to that received by animals exposed to "sunshine." By shielding the thermopile from the direct solar rays it is possible to measure the intensity of skyshine, and thus determine the part it plays in furnishing antirachitic energy.

Application of these data to the experiments in progress during these two months shows that the rats in experiment 27 (table 1) received an average total radiation (sunshine + skyshine), of wave lengths shorter than $313\text{ m}\mu$, equivalent to $0.000407\text{ gm. cal. per sq. cm. per min.}$, or $0.00073\text{ gm. cal. per sq. cm. daily}$, of which $0.00061\text{ gm. cal. per sq. cm.}$ was direct sunshine. In addition (see table 1) the animals received an average of 2.2 minutes of skyshine daily (in the absence of direct sunshine). The average total radiation of less than $313\text{ m}\mu$ in skyshine for this period was $0.000290\text{ gm. cal. per sq. cm. per min.}$ The sum of these values, the total amount of antirachitic radiation which the rats received, was therefore $0.00137\text{ gm. cal. per sq. cm. daily}$. The skyshine group in this series received an average daily exposure of 11.8 minutes (table 4) which is equivalent to $0.0034\text{ gm. cal. per sq. cm. of wave lengths less than }313\text{ m}\mu$. Similar treatment of the data for experiment 28 shows that the animals in the sunshine group received a total of $0.0012\text{ gm. cal. per sq. cm. daily}$ of wave lengths less than $313\text{ m}\mu$ as compared to the skyshine group which received $0.0037\text{ gm. cal. per sq. cm. daily}$. Similarly, the sunshine (sunshine + skyshine) group of chicks exposed during this period (expt. 18, table 2) was receiving radiation, of wave lengths shorter than $313\text{ m}\mu$, equivalent to $0.000449\text{ gm. cal. per sq. cm. per min.}$, or $0.00090\text{ gm. cal. per sq. cm. daily}$, from the sun and the sky, and in addition an average of 1.8 minutes daily of skyshine radiation alone, equivalent to $0.000244\text{ gm. cal. per sq. cm. per min.}$, a total of $0.00134\text{ gm. cal. per sq. cm. daily}$. In this experiment the group exposed to skyshine received a total of $0.00289\text{ gm. cal. per sq. cm. daily}$. It is quite evident that the exposures to sky-

shine in each case could have been reduced by at least one-half and would still have been adequate.

These calculations indicate that antirachitic energy equivalent to a total of from 0.0420 to 0.0490 gm. cal. per sq. cm. (0.0012 to 0.0014 gm. cal. per sq. cm. daily for 35 days) can be considered as the amount which will prevent the appearance of rickets in rats, while 0.0563 gm. cal. per sq. cm. (0.00134 gm. cal. per sq. cm. for 42 days) is more than adequate protection for chicks. That the value of 0.0490 gm. cal. per sq. cm. represents the approximate minimal energy requirement for rats when no wave lengths shorter than 300 $m\mu$ are present is shown by additional experiments in which rats were exposed to this and to smaller amounts of radiation from a flaming carbon arc (25-28 A; 55-60 V; Sunshine Carbons) and from a quartz mercury vapor lamp (5-6 A; 70-80 V).

Three sets of experiments were performed in which the rats were exposed 1, to the unscreened radiation of the arcs; 2, to radiation longer than 290 $m\mu$, the arcs being screened by a Corex D filter; 3, to radiation longer than

TABLE 6

Antirachitic energy content of flaming carbon and quartz mercury vapor arcs at 1 M, in gm. cal. per sq. cm. per min.

	SHORTER THAN 319 $m\mu$	290-319 $m\mu$	300-319 $m\mu$
Pan Ray Arc 25-28 A; 55-60 V; Sunshine Carbons.....	0.00359	0.00213	0.00142
Quartz Hg Vapor Arc 5-6 A; 70-80 V.....	0.00427	0.00221	0.00122

300 $m\mu$, Pyrex glass being used as a filter. The wave lengths included in the second set represent those found in sunshine under ideal conditions, 290 $m\mu$ being considered the approximate wave length limit of solar radiation, while the wave lengths included in the third set are those previously indicated as having been present under the conditions of our experiments. The antirachitic energy content and distribution of the two sources was measured spectroradiometrically with a Hilger monochromatic illuminator (table 6) and the rats exposed weekly for lengths of time so calculated (proper corrections being made for the percentage wave length transmission of the filters used) as to provide a total radiation of 0.0490 gm. cal. per sq. cm. over a period of five weeks.

Complete protection was obtained with this amount of energy in the first two sets of experiments in which the rats were exposed to the unfiltered radiation of the carbon and quartz mercury vapor arcs and to the radiation filtered through Corex D. Radiation equivalent to one half this amount prevented rickets in the groups exposed to the quartz mercury vapor arc, but only partially protected those exposed to the carbon arc. In the third

set of experiments in which the rats were irradiated through Pyrex glass (in the absence of wave lengths shorter than $300\text{ m}\mu$), those exposed to the carbon arc radiation showed no rickets, while several of the animals exposed to the quartz mercury vapor lamp showed mild rickets. Shorter exposures resulted in only partial or no protection in both groups. Differences in the spectra of the two arcs account for the variation in the results, the energy in the carbon arc being chiefly of wave lengths longer than $300\text{ m}\mu$, while the mercury vapor lamp has the powerful antirachitic band at 297 to $302\text{ m}\mu$ but relatively little effective antirachitic energy of longer wave lengths, the line at $313\text{ m}\mu$ being only slightly antirachitic.

SUMMARY

Exposure of rats and chicks on rickets-producing diets over periods of 31 and 27 months, respectively, shows that an average daily exposure to New Orleans sunshine (sun + sky) of from 2 to 3 minutes from April through October prevents the appearance of rickets, as do average daily exposures of from 5 to 6 minutes from November through January. Average daily exposures of chicks for from 3 to 5 minutes, and of rats for from 5 to 6 minutes are antirachitic during February and March.

Average daily exposures to skyshine of from 16 to 17 minutes from May through September prevent rickets in rats, as do average daily exposures from 22 to 28 minutes from October through February, and from 16 to 20 minutes during March and April. Proper calcification and growth is obtained in chicks exposed to skyshine with average daily exposures of from 6 to 11.4 minutes from July through October; of 35 minutes from November through February; and of from 18 to 19 minutes from March through June. Rats and chicks exposed under Vitaglass, Corex D and Cel-O-Glass to sunshine (sunshine + skyshine) or to skyshine are protected throughout the year by durations of exposure correspondingly greater in proportion to their relative absorption.

The average daily amount of direct solar radiation of wave lengths shorter than $313\text{ m}\mu$ which will prevent rickets in rats is equivalent to less than 0.001 gm. cal. per sq. cm. ($70\text{ }\mu\text{w}$) from April to October, and between 0.001 and 0.002 gm. cal. per sq. cm. during the rest of the year. The minimum amount of solar energy of wave lengths shorter than $313\text{ m}\mu$, effective in preventing leg weakness and promoting growth in chicks was found to be about 0.00069 gm. cal. per sq. cm. ($48.3\text{ }\mu\text{w}$) daily. Measurement of the total antirachitic radiation, which includes the additional skyshine to which the animals are exposed, shows that in June and July antirachitic radiation from sunshine and skyshine equivalent to between 0.042 and 0.049 gm. cal. per sq. cm. (0.0012 ($84\text{ }\mu\text{w}$) to 0.0014 ($98\text{ }\mu\text{w}$) gm. cal. per sq. cm. daily for 35 days) will prevent rickets in rats and a total of 0.0563 gm. cal. per sq. cm. (0.00134 gm. cal. per sq. cm. for 42 days) will provide for normal calcification and growth of chicks.

Experiments in which rats were exposed to carbon and quartz mercury vapor are radiation equivalent to 0.049 gm. cal. per sq. cm. demonstrate that this value can be considered as the approximate minimum amount of energy needed to prevent rickets in rats when no wave lengths shorter than 300 m μ are present, and affords, therefore, more than adequate protection when shorter wave lengths are present.

BIBLIOGRAPHY

- CLARK, J. H. 1931. *Journ. Opt. Soc. Am.*, iv, 240.
- COBLENTZ, W. W., R. STAIR AND J. M. HOGUE. 1931. *Bur. Stand. Journ. Res.*, vii, 723.
- CRAWFORD, R. AND C. R. WILLIAMSON. 1930. *New Orleans Med. and Surg. Journ.*, lxxxiii, 219.
- DAY, P. L. 1932. *Amer. Journ. Dis. Child.* (in press).
- DEBUYS, L. R. 1924. *Amer. Journ. Dis. Child.*, xxvii, 149.
- DEBUYS, L. R. AND L. VON MEYSENBUG. 1924. *Amer. Journ. Dis. Child.*, xxviii, 327.
- DICK, J. L. 1922. *Rickets*.
- DORNO, C. 1919. *Physik der Sonnen und Himmelsstrahlung*.
- GODFREY, L. S. 1931. *Journ. Prev. Med.*, v, 1.
- GORCZYNSKI, L. 1924. *Journ. Opt. Soc. Am.*, ix, 455.
- HESS, A. F. 1925. *Journ. Amer. Med. Assoc.*, lxxxiv, 1033.
- KIMBALL, H. H. AND H. E. HOBBS. 1923. *Journ. Opt. Soc. Am.*, vii, 707.
- LEWIS, R. C., G. M. FRUMESS AND H. B. STEIN. 1931. *Amer. Journ. Dis. Child.*, xli, 71.
- RUSSELL, W. C. AND C. H. HOWARD. 1931. *Journ. Biol. Chem.*, xci, 493.
- SLOAN, H. S. 1929. *Thesis* (unpublished).
- STEIN, H. B. AND R. C. LEWIS. 1931. *Amer. Journ. Dis. Child.*, xli, 62.
- TISDALL, F. F. AND A. BROWN. 1929. *Journ. Amer. Med. Assoc.*, xcii, 860.
1931. *Amer. Journ. Dis. Child.*, xlii, 1144.
- WILLIAMS, C. T. 1928. *Amer. Journ. Dis. Child.*, xxxv, 357.

ALTERATIONS IN BLOOD LACTIC ACID AS A RESULT OF EXPOSURE TO HIGH OXYGEN PRESSURE

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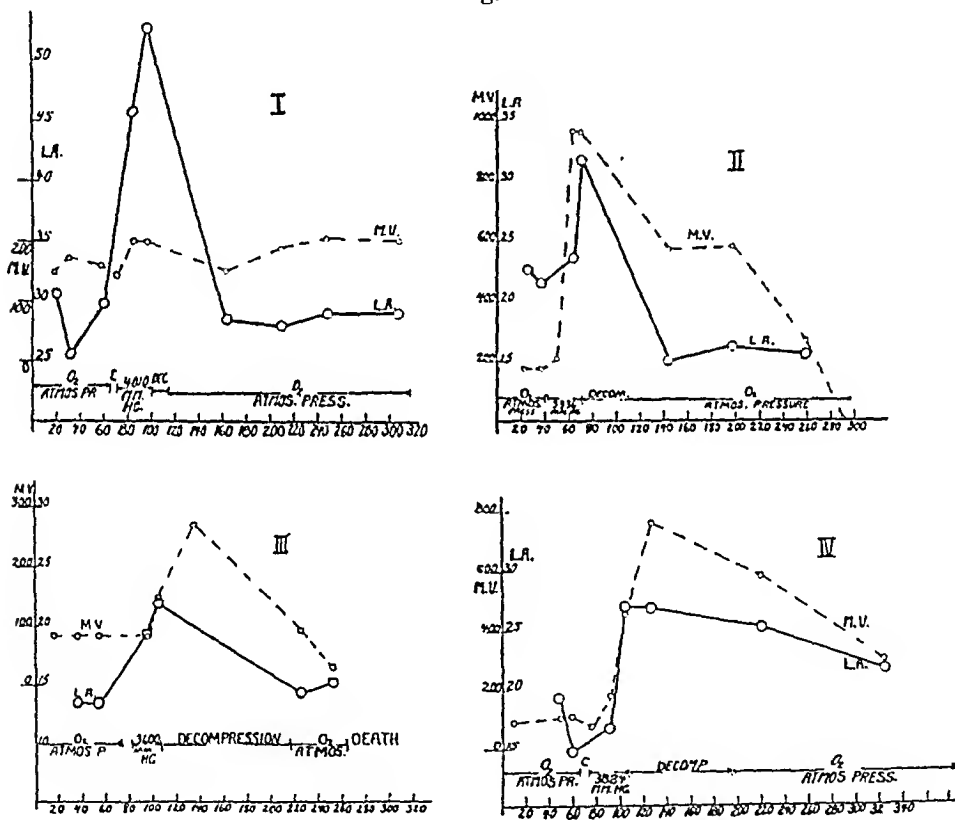
The removal of lactic acid from the mammalian organism is essentially an aerobic process. We might suppose then that in the presence of an abundance of oxygen the lactic acid content of the organism would be at a minimum. There is evidence however that with exposure of an animal to high oxygen pressures, oxidations are diminished. Might this diminution be reflected in the lactic acid content of the organism? The present series of experiments was carried out to determine the alterations in the lactic acid content of the blood of animals exposed to several atmospheres of oxygen. It is of course understood that the changes in the blood lactic acid do not necessarily constitute an infallible criterion of the changes in the total lactic acid content of the organism.

The animals used in our experiments were dogs weighing from eight to twelve kilograms, anesthetized with morphine and urethane. In all but a few of the experiments anti-coagulant (heparin) was used. The pressure chamber with the accessory apparatus was that described earlier (Bean, 1931). It provided means of recording graphically pulmonary ventilation, blood pressure and pulse rate. Blood lactic acid was determined by the Friedemann, Cotonio and Schaffer method (1927). The blood from which the samples were taken circulated continuously through a glass tube connecting the central and peripheral ends of one carotid artery. This tube in a part of its course ran outside of the pressure chamber and was there provided with a stopcock through which the blood samples were taken with the aid of a syringe. Intravenous injection with the animal under pressure was made possible by connecting the lower end of a burette to a stopcock through the chamber wall and to the femoral vein; the upper end was led back into the chamber by pressure tubing and a second stopcock.

The experimental animal first breathed pure oxygen at atmospheric pressure for about thirty or forty minutes. The pressure was then rapidly raised to about five atmospheres and maintained at this level for approximately twenty minutes. Decompression was carried out in stages and over a period of such duration as to avoid effervescence which might

result from too rapid decompression. Following decompression the animal breathed oxygen at atmospheric pressure to the end of the experiment. Blood samples for lactic acid determinations were taken before, during and after exposure to the high pressure. These samples in most instances were twelve cubic centimeters. In order to obviate so far as possible any deleterious effects which the loss of blood might entail, an equal volume of saline was injected intravenously after the withdrawal of each sample.

Fig. 1



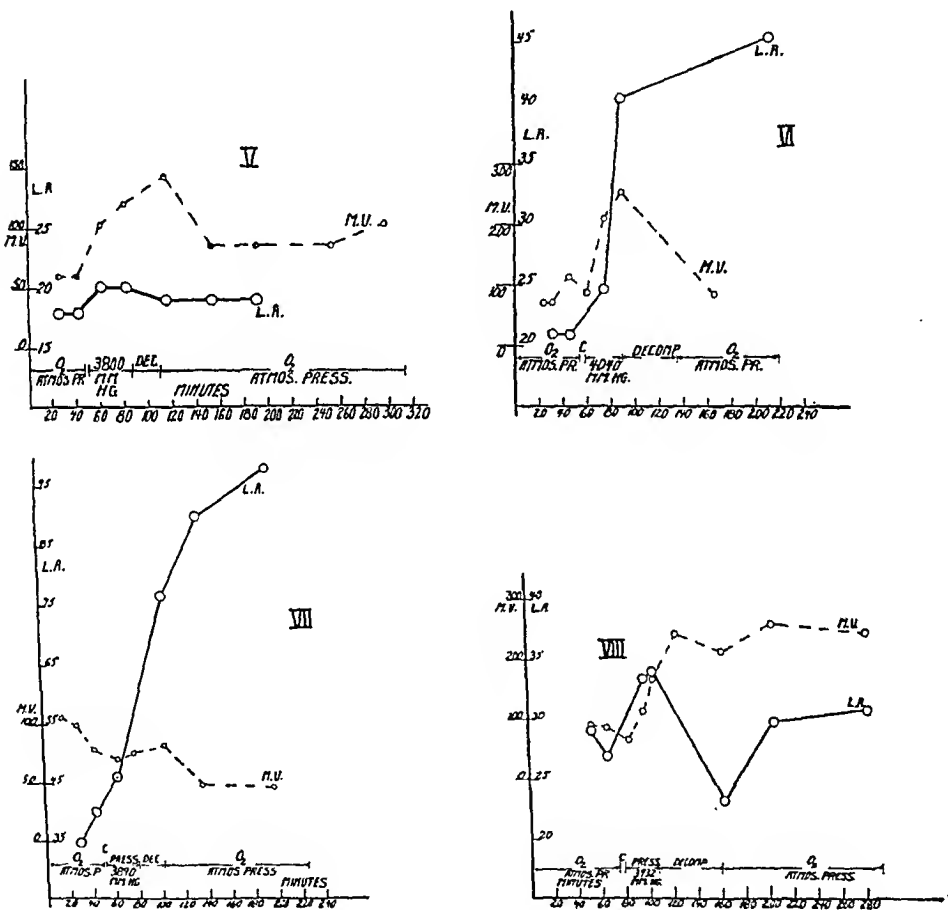
The M. V. figures on the ordinate represent one-tenth the total respiratory minute volume in cubic centimeters.

Breathing pure oxygen at one atmosphere was associated with little or no change in the lactic acid content of the blood. The change if any was usually a decrease. During the periods of exposure to high pressure the blood samples in fifteen of the nineteen experiments constituting this series showed an increase in lactic acid and in four they showed no appreciable change. Of those experiments in which there occurred an increase in the blood lactic acid, six showed complete recovery in so far as lactic acid was concerned, during, or subsequent to decompression. In six others recovery

was evident but incomplete and in the remaining three the blood lactic acid continued to rise through the decompression period and to the death of the animal.

The respiratory minute volume increased during exposure to the high pressure in all the experiments except one, in which there occurred a slight decrease.

Fig. 2



The M. V. figures on the ordinate represent one-tenth the total respiratory minute volume in cubic centimeters.

These changes are illustrated in figures 1 and 2, in which blood lactic acid in milligrams per cent and respiratory minute volume are plotted on the ordinates against time in minutes on the abscissae. The duration of the periods of exposure to the various pressures are indicated along the abscissae. Curve I shows an initial drop in lactic acid during exposure to oxygen at one atmosphere and a return to the original value before

compression was begun. While under an oxygen pressure of 4000 mm. Hg the lactic acid rose from 30 to 54 mgm. per cent; with decompression it returned to the precompression value. Curve II also indicates a preliminary decrease in the blood lactic acid but in this case it approaches the limit of experimental error. During exposure to the increased pressure, which in this instance was 3336 mm. Hg the lactic acid rose appreciably. On decompression and subsequent breathing of oxygen at one atmosphere the lactic acid content of the blood fell to values decidedly below those of the precompression period. An initial drop in blood lactic acid is shown also in curve VIII, figure 2, although it subsequently rose above the precompression level during exposure to oxygen at a pressure of 3932 mm. Hg. A recovery decrease in lactic acid was well marked in this experiment but not sustained. In curve III the initial fall is absent but the increase under pressure and subsequent complete recovery are present as in curves I and II. Curve IV of figure 1 illustrates another experiment in which there occurred an initial fall in the blood lactic acid while the animal was breathing oxygen at one atmosphere pressure and a rise during the high pressure period. The recovery however was neither so complete nor as rapid as in the aforementioned curves. In curve V there is a definite though less pronounced increase in blood lactic acid. Curve VI is illustrative of those experiments in which the lactic acid of the blood continued to rise even after decompression. Curve VII represents an exception in that breathing oxygen at one atmosphere was attended by an increase in the lactic acid of the blood. There was moreover no indication of any recovery from the lactic acid increase after decompression. This experiment was exceptional also in that the respiratory minute volume decreased generally throughout the experiment. It may have been that the animal was in poor condition to begin with, although the blood pressure and the pulse rate did not indicate that such was the case.

The changes in respiratory minute volume consequent upon exposure to high pressures of oxygen used in our experiments are evident from the graphs of figures 1 and 2 (the broken line curves). That breathing oxygen at one atmosphere gives rise to little or no change in the respiratory minute volume finds substantiation in our curves. The increase in ventilation which is attendant upon exposure to high pressures of oxygen is what might be expected considering the earlier findings in this respect of Bert (1878), Smith (1899), Hill and Macleod (1903), Gesell (1923), and Bean (1929). An explanation of this typical respiratory response in oxygen poisoning, based on a broken coordination of the dual function of hemoglobin, has been given by Gesell (1923), and supported by experimental evidence (Bean, 1931). The increase in ventilation was well marked in most of our experiments even though the duration of exposure to high pressure was relatively short.

DISCUSSION. The lactic acid increase in these experiments might conceivably have been due to any one or several of a number of factors which are involved in its metabolism and its shift within the organism.

It has been well established that there is a large increase in lactic acid during severe muscular exercise. At first sight the curves in figures 1 and 2 might suggest that in our experiments the blood lactic acid increase was due to an augmented activity of the respiratory muscles during exposure to the increased oxygen pressure. That this could not have been the primary cause for the increased blood lactic acid may be concluded from a close inspection of these curves. During the breathing of oxygen at one atmosphere (curve I, fig. 1) there is an inverse relationship between the respiratory minute volume and the blood lactic acid. Moreover the relatively small increase in the respiratory movements during exposure to 4000 mm. Hg pressure would in itself be insufficient to cause the attendant large increase in the blood lactic acid. In curve III of the same figure it is to be noted that while the lactic acid content of the blood increased from 12 to 20 mgm. per cent for the first sample taken under the increased oxygen pressure, the respiratory minute volume remained unaltered. In other words, the lactic acid increase preceded the respiratory minute volume increase. Again, in curve IV, figure 1, there is to be seen an inverse relationship between the lactic acid content of the blood and ventilation during the initial period of breathing oxygen at atmospheric pressure. In curve V, figure 2, the ventilation continued to increase while the blood lactic acid decreased on decompression. These instances of a lack of parallelism between blood lactic acid and ventilation support the belief that the rise in lactic acid was not due primarily to increased muscular movement. Moreover there are other conditions which bring about marked hyperpnea without any accompanying increase in the blood lactic acid. Administration of carbon dioxide gives rise to increased activity of the respiratory muscles but the lactic acid content of the blood is decreased. (Gesell, Krueger, Gorham, and Bernthal, 1930.)

Several experiments were performed to determine whether heparin as used in our procedure had any effect on blood lactic acid. Essentially the same results were obtained with and without the use of heparin from which it was concluded that heparin was not a determining factor in the increase in blood lactic acid in our experiments.

The accumulation of blood lactic acid might have been due to its inadequate removal. This removal, barring elimination by way of the skin and kidneys, is an aerobic process. During exposure to high pressures of oxygen, as used in our experiments, the blood is saturated with oxygen (Bean, 1931). Any failure in the removal of lactic acid cannot therefore be attributed to a lack of oxygen. It is possible, however, that even in the presence of an abundance of oxygen, the oxidative mechanisms of the

tissues, notably the liver, which remove lactic acid from the blood might be impaired as a result of oxygen poisoning. In numerous pathological conditions involving injury of the liver cells such as phosphorus poisoning, carcinoma, fatty degeneration and acute yellow atrophy there is a concomitant increase in blood lactic acid. (Büttner, 1926; Valentin, 1925; Noah, 1927; Schumacher, 1926.)

There is evidence that in oxygen poisoning there is a decrease in oxidations (Hill and Macleod, 1903; Bean, 1931). It would appear therefore that the accumulation of lactic acid under high oxygen pressure might be due to a decrease in the oxidative removal rather than to an increase in the rate of production of lactic acid. In this connection it is of interest to note that following exposure to high oxygen pressure a temporary (possibly compensatory) overshooting of oxygen absorption was usually observed (Bean, 1931). The conditions produced by oxygen poisoning might be similar to those resulting from injection of cyanide. In animals injected with sodium cyanide there is every reason to believe that the blood is fully oxygenated yet oxidations decreased and lactic acid increased in the blood. Here with uniform ventilation there was also a tendency toward overshooting of oxygen consumption during recovery. (Gesell, Krueger, Gorham and Bernthal, 1930.)

In considering the increase in blood lactic acid another factor should be taken into account, namely, a broken coördination of the dual function of hemoglobin, which occurs at pressures of oxygen above three atmospheres (Bean, 1929). Under these pressures the tissues obtaining plenty of oxygen for their immediate needs from that in solution in the plasma leave the hemoglobin unreduced. As a result no base is supplied for the transport of carbon dioxide to the lungs. The carbon dioxide accumulates in the tissues turning them acid and this increased acidity is shortly reflected in the blood. Campbell (1930) has found an accumulation of carbon dioxide in tissues of animals exposed to high oxygen pressure. Increased acidity inhibits oxidations. It is possible therefore that in oxygen poisoning the decreased oxygen absorption is due in part at least to the increase in acidity arising from the accumulation of carbon dioxide. The lactic acid accumulation might in turn be attributed to the lowered oxidations. This increase in lactic acid content of the blood would emphasize the acidosis. In this connection it is to be noted that it is not uncommon for the hyperpnea resulting from exposures to high pressures of oxygen to diminish quite abruptly to the point of apnea. This response might be explained as being due to a rapid fatigue of the respiratory control mechanism under the influence of the intracellular acidity which is increasing rapidly, not alone because of the accumulation of carbon dioxide but also because of fixed acids in the centers.

There are however numerous examples in which the relation of acidity,

oxidations and lactic acid content of the blood as suggested above does not exist. It has been found that intravenous injections of sodium bicarbonate give rise to an increase in blood lactic acid (Macleod and Hoover, 1917; Anrep and Cannon, 1923; Gesell, Krueger, Gorham and Bernthal, 1930b) and at the same time an increase in oxygen consumption. Intravenous injection of hydrochloric acid increased the hydrogen ion concentration of the blood, decreased the blood carbon dioxide capacity and the blood lactic acid. Administration of gas mixtures with a high carbon dioxide content (11.42 per cent) diminished oxidations and commonly lowered blood lactic acid. From the results of these experiments in which carbon dioxide was administered with constant artificial ventilation it was concluded that "it would thus appear that neither rate of oxidations nor blood and tissue acidity were the primary determining factors controlling the lactic acid equilibrium under these particular conditions." (Gesell, Krueger, Gorham and Bernthal, 1930a.)

It is conceivable that an accumulation of carbonic acid in the tissues might act to increase the lactic acid in the blood not necessarily as a result of an alteration in the rate of its formation or of its removal but because of a shift from the tissues to the blood. Such a shift has been proposed as a possible explanation for an increase in blood lactic acid under other experimental conditions (Gesell, Krueger, Gorham and Bernthal, 1930).

The accumulation of lactic acid in the presence of an abundance of oxygen suggests that oxidations are regulated by some factor other than the presence of oxidizable materials and sufficient oxygen. Might this factor be of the nature of an enzyme? If so we might explain the decreased oxidations (Bean, 1931) and consequent lactic acid accumulation during exposure to high oxygen pressure as being due to a poisoning of this enzyme. Recovery from the increased blood lactic acid on decompression might indicate a return of enzymatic function or a production of fresh unpoisoned enzyme. In support of this hypothesis we have during the recovery period a reduction in blood lactic acid and, as previously reported (Bean, 1931), a temporary overshooting of oxygen absorption. With a return to normal enzymatic control we would expect such increased oxidations in the presence of an abundance of oxygen and oxidizable materials. Failure of recovery might indicate an irreversible alteration in the enzymes or a permanent damage to cells involved in the production of enzymes. Perhaps a similar explanation for the effects produced by intravenous injection of sodium cyanide might not be amiss.

SUMMARY

The purpose of these experiments was to determine alterations in the content of blood lactic acid which might occur during exposure of animals to oxygen at pressures of about five atmospheres.

The experimental animals were dogs anesthetized with morphine and urethane. Periods of exposure to the increased pressure were usually of about thirty minutes' duration and were preceded and followed by administration of oxygen at one atmosphere. Decompression was carried out in stages, and over such periods of time as to avoid effervescence which might occur with too rapid decompression.

The preliminary exposure to oxygen at one atmosphere was frequently attended by a slight drop in the lactic acid content of the blood.

Subsequent breathing of oxygen at pressures of about five atmospheres usually was attended by an increase in blood lactic acid. Fifteen of nineteen experiments showed an increase in blood lactic acid while four showed no appreciable change. Recovery from the lactic acid increase was practically complete in six experiments on decompression. Six others showed only partial recovery and in three more the lactic acid continued to increase during and subsequent to decompression.

Respiratory minute volume was increased during exposure to high oxygen pressure in all experiments except one.

Evidence was presented to show that the lactic acid increase was not due to increased activity of the respiratory muscles.

Cellular damage resulting from high oxygen pressure was considered as a possible factor contributing to the lactic acid increase in the blood.

Intracellular acidity arising from a broken coördination of the dual function of the hemoglobin was discussed as a possible indirect cause for the lactic acid increase. The increased intracellular acidity might give rise to an augmented production of lactic acid by lowering of oxidations. It might also cause a shift in lactic acid from the interior of the cell to the blood.

The possibility of an enzymatic control of lactic acid removal was suggested. High oxygen pressure might have a toxic effect on the enzymes involved thereby accounting for the accumulation of lactic acid during exposure of the animal to high oxygen pressure.

BIBLIOGRAPHY

- ANREP, G. V. AND R. K. CANNON. 1923. *Journ. Physiol.*, lviii, 244.
BEAN, J. W. 1931. *Journ. Physiol.*, lxxii, 28.
BERT, P. 1878. *La Pression Barométrique*. Paris.
BÜTTNER, H. E. 1926. *Klin. Wochenschr.*, v, 1507.
CAMPBELL, J. A. 1930. *Journ. Physiol.*, lxviii, Proc., vii.
FRIEDMANN, T. E., M. COTONIO AND P. A. SCHAFER. 1927. *Journ. Biol. Chem.*, lxxiii, 335.
GESELL, R. 1923. *This Journal*, lxvi, 5.
GESELL, R., H. KRUEGER, G. GORHAM AND T. BERNTHAL. 1930a. *This Journal*, xciv, 402.
1930b. *This Journal*, xciv, 387.

- HILL, L. AND J. J. R. MACLEOD. 1903. Journ. Hyg., iii, 401, 420.
MACLEOD, J. J. R. AND D. H. HOOVER. 1917. This Journal, xlii, 460.
NOAH, G. 1927. Klin. Wochenschr., vi, 1465.
SCHUMACHER, H. 1926. Klin. Wochenschr., v, 497.
SMITH, J. L. 1899. Journ. Physiol., xxiv, 20.
VALENTIN, F. 1925. Münch. Med. Wochenschr., lxxii, 86.

STUDIES ON THE KINETICS OF LACTATE FORMATION IN MUSCLE UNDER THE INFLUENCE OF IODOACETIC ACID

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Since the original observation by Lundsgaard (1930) that iodoacetic acid prevents the accumulation of lactate in striated muscle with only partial depression of excitability and contractility, considerable interest has been directed toward the possible mode of action of the substance. Dudley (1931) has found that iodoacetic acid inhibits the normal conversion of phenyl-glyoxal to mandelic acid by glyoxalase. Lundsgaard (1930) observed an accumulation of methyl-glyoxal in muscle following iodoacetic acid poisoning, and Case and Cook (1931), by means of fixation by sodium sulfite, have detected an accumulation of both methyl-glyoxal and pyruvic acid in normal muscle. Lundsgaard (1930) observed that glycogen breakdown progresses normally in iodoacetic acid treated muscle, but that hexose-diphosphoric acid ester accumulates. Although the mass of evidence seemed to point conclusively toward an inhibition of carbohydrate breakdown prior to the lactic acid stage, it occurred to the authors that one possible mode of alteration of the carbohydrate cycle, not previously investigated, had not been entirely ruled out by previously recorded observations.

The fact that hexose-diphosphoric acid ester accumulates in iodoacetic acid treated muscle in amount sufficient to have suggested to Lundsgaard (1930) the possibility of a stabilization of that substance, presented the possibility that iodoacetic acid might effect the removal of the lactic acid formed during the contraction by accelerating its conversion to hexose-diphosphoric acid ester in the early post-contraction period. During the investigation of this problem a number of interesting facts have come to light. These have to do particularly with the kinetics of lactic acid production in the presence of iodoacetic acid.

All intact muscles were frozen by immersion in liquid air before preparation of the protein-free filtrate. Precipitation of proteins was accomplished by the use of tungstic acid, by a procedure previously described (Visser and Smith, 1930). Lactic acid was determined by the method of Friedemann, Cotonio, and Shaffer (1927).

Minced muscle was prepared by thorough grinding with sand in a mortar. Mono-iodoacetic acid, Eastman, was used, unneutralized, throughout, since the concentra-

tion was lower in most cases than that used by other workers, and too low to affect the pH significantly. Merck lactic acid, 85 per cent, was diluted for use in those experiments requiring addition of lactic acid. Stimulation was uniformly done directly, in oxygen-free Ringer by means of condensor discharge usually spaced to allow an interval of 1 second.

1. *The fate of preformed or added lactic acid in iodoacetic-acid treated muscle.* If the effect of iodoacetic acid were an acceleration of lactate removal, it seemed reasonable to us to assume that lactic acid would be taken up from a lactic-acid bearing solution and converted into a hexose-phosphoric acid ester by iodoacetic acid treated muscle.

TABLE 1
Influence of iodoacetic acid upon added lactic acid

EXPERIMENT	IDIOACETIC ACID ADDED	LACTIC ACID ADDED	LACTIC ACID BY ANALYSIS		TIME IN SOLUTION	TOTAL LACTIC ACID BY ANALYSIS—MUSCLE PLUS SOLUTION
			Muscle	Solu- tion		
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>mgm.</i>
1 { A (gastroc.) B (gastroc.)	1/20,000	0.039	0.045	0.038	4:00- 5:20	2.213
	0	0.039	0.049	0.036		2.192
2 { A (sart.) B (sart.)	1/30,000	0.039	0.041	0.036	4:45- 5:45	2.040
	0	0.039	0.061	0.040		2.315
3 { A (gastroc.) B (gastroc.)	1/10,000	0.039	0.040	0.038	5:10- 7:50	2.435
	0	0.039	0.042	0.039		2.462
7 { A (sart.) B (sart.)	1/30,000	0.039	0.049	0.039	10:02-11:15	2.205
	0	0.039	0.057	0.036		2.133
8 { A (gastroc.) B (gastroc.)	1/10,000	0.039	0.042	0.037	10:15-12:00	2.870
	0	0.039	0.043	0.036		2.855

Consequently, pairs of normal sartorius and gastrocnemius muscles from small winter frogs were placed in Ringer solution containing 0.039 per cent of lactic acid, and in the case of one muscle from each pair from 0.01 per cent to 0.0033 per cent of iodoacetic acid. Both muscles of a pair and the solutions in which they had been immersed were prepared for analysis after intervals varying from 60 to 160 minutes (table 1).

The findings do not indicate any significant variation between the total lactic acid content of the iodoacetic acid containing system and that of the iodoacetic-acid-free system. The lactic acid content of any individual muscle and the Ringer in which it had been immersed indicates an approximate diffusion equilibrium if account be taken of the inescapable water loss from the muscle to the slightly hypertonic immersion fluid. The use of

commercial, racemic lactic acid might be questioned, except for the fact that in all cases that lactic acid content of the iodoacetic acid containing system should have been the lower had the not insignificant amount of pre-formed sarco-lactic acid (the "resting" content) been resynthesized or otherwise removed. In a single experiment, an attempt to test the latter point, both muscles of a similar pair were fatigued, one placed in iodoacetic acid Ringer and the other in plain Ringer. At the end of a two-hour period the lactic acid content of each was essentially the same.

It occurred to us that a more crucial test of the problem might consist of the use of muscle poisoned by injection of iodoacetic acid into the intact animal. To this end iodoacetic acid in concentration of 0.165 mgm. per gram, based on the gross weight of the frog, was injected into the ventral

TABLE 2

Influence of iodoacetic acid upon the final concentration of lactate in macerated poisoned muscle

EXPERIMENT	TYPE AND WEIGHT OF MUSCLE	INCUBATION PERIOD	LACTIC ACID ADDED	LACTIC ACID FOUND	IDOACETIC INJECTED
		hour	mgm.	mgm.	
22 {	1 Thigh, 2.96 gram	2	10	9.42	1/6,000
	2 Thigh, 2.80 gram	0	10	9.78	
23 {	1 Thigh, 3.51 gram	0	10	9.25	1/6,000
	2 Thigh, 3.43 gram	2	10	10.10	

Calculated amount present if lactate accumulation had proceeded unchecked, on the basis of 0.5 per cent as an average final lactate concentration.

22, 1..... 24.80 mgm.

23, 2..... 27.15 mgm.

lymph sac, and thigh muscle taken after 40 minutes. Muscle from one thigh of a single animal was frozen immediately and its proteins precipitated after the addition to the system of 10 mgm. of lactic acid in 10 cc. of Ringer. The corresponding muscles of the opposite thigh were ground with sand in a mortar, 10 mgm. of lactic acid in 10 cc. of Ringer added, and the mixture subjected to two hours' incubation at room temperature. Within the limits of the error introduced through the difficulty of quantitative transfer from the mortar while holding the total volume low, the final total lactate concentration is essentially the same in the two cases. Two typical experiments are shown in table 2. It is noticeable that no new lactate accumulation took place in the muscle itself after this treatment. The calculation appended to table 2 shows this in a striking manner.

2. *The effect of concentration of iodoacetic acid on irritability, and upon lactate accumulation in intact and minced muscle.* Since we had obtained

apparently complete inhibition of lactate production by the comparatively low concentration of 0.165 mgm. per gram, it seemed important to us to make an attempt to determine the liminal effective concentration of iodoacetic acid.

TABLE 3
Influence of concentration of iodoacetic acid upon lactate production

EXPERIMENT	IDOACETIC INJECTED	TIME AFTER INJECTION	PROCEDURE	LACTIC ACID FOUND
		<i>minutes</i>		<i>per cent</i>
27 {A	1/5,000	37	Resting	0.019
B			Fatigued	0.013
22 {A	1/6,000	40	Fatigued	0.020
B			Fatigued	0.023
29 {A	1/15,000	35	Resting	0.015
B			Fatigued	0.019
30 {A	1/15,000	45	Resting	0.017
B			Fatigued	0.021
32 {A	1/15,000	60	Resting	0.029
B			Fatigued	0.031
33 {A	1/15,000	60	Resting	0.016
B			Fatigued	0.029
34 {A	1/20,000	30	Resting	0.028
B			Fatigued	0.033
35 {A	1/20,000	30	Resting	0.032
B			Fatigued	0.028
31 {A	1/30,000	30	Resting	0.091
B			Fatigued	0.195
		<i>hours</i>		
9 {A	1/30,000	2	Unstimulated	0.035
B	1/30,000	2	Stimulated	0.122
10 {A	1/30,000	2 $\frac{1}{4}$	Unstimulated	0.022
B	1/30,000	2 $\frac{1}{4}$	Stimulated	0.119

To this end frogs were injected with concentrations of iodoacetic acid varying from 0.2 mgm. per gram to 0.033 mgm. per gram, and dissection of the gastrocnemius muscles made following intervals of from 30 to 135 minutes. One muscle of each pair was frozen immediately as a control, its mate stimulated to fatigue and frozen subsequently. Concentrations of

iodoacetic acid of 0.05 mgm. per gram and above uniformly allowed no lactate accumulation, while 0.033 mgm. per gram showed incomplete inhibition of lactate production (table 3).

Tissue irritability did not appear to correspond as closely to the concentration of iodoacetic acid as did the inhibition of lactate production, muscles from animals injected with 0.033 mgm. per gram occasionally showing complete depression of irritability, and muscles from animals receiving high concentrations frequently responding to stimuli. Lactate accumulation was quite constantly inhibited, however, in concentrations above 0.033 mgm. per gram. The depression of irritability raised a further significant question. Could not the depression of irritability and the feeble response to stimuli be responsible for the low lactate accumulation in poisoned muscles? A further possibility also presented itself. Might

TABLE 4
Lactate accumulation in chloroform rigor

EXPERIMENT	IDOACETIC INJECTED	INTERVAL BE- TWEEN INJECTION AND DISSECTION	LACTIC ACID FOUND	
			<i>per cent</i>	
23 {A	1/6,000	10:45-11:45	0.013	Muscle not irritable
B	1/6,000	10:45-11:45	0.014	Chloroform rigor
28 {A	1/5,000	3:03- 3:50	0.019	Muscle not irritable
B	1/5,000	3:03- 3:50	0.034	Chloroform rigor
Control			0.020	Resting
Control			0.509	Chloroform rigor
Control			0.490	Chloroform rigor

not the reconversion of lactic acid to hexose phosphate, if such occurred, proceed at such a rate that our unavoidable delay in freezing and precipitating made it impossible for us to determine the lactic acid actually formed? Subjecting muscle to treatment such as chloroform rigor, with the consequent rapid great increase in lactic acid, independent of natural irritability, should offer an opportunity to test both of these questions. Freshly dissected gastrocnemius muscles from poisoned frogs were subjected to the action of chloroform and analyzed in the usual manner. Lactate accumulation did not occur with such treatment as is evident from table 4, in which is also indicated the high lactate accumulation normally accompanying this procedure. It seems conclusive then that iodoacetic acid in high concentrations inhibits lactate accumulation, and does so quite independently of natural irritability. Furthermore, it becomes more probable that delay incident to the manipulation of freezing in liquid air is not a factor in masking lactate formation by allowing its removal.

It seemed advisable, however, to study the effect of concentration in another, quite different, type of experiment, namely, in minced muscle. Frogs were injected as before with varying concentrations of iodoacetic acid, from 0.2 mgm. per gram to 0.033 mgm. per gram. Thigh muscle from an individual animal was grossly minced with scissors and shuffled thoroughly, and in most cases divided into three portions. One large portion was weighed, ground with a few cubic centimeters of Ringer in a mortar without having been previously frozen, and set aside for incubation at room temperature. A second portion was frozen and prepared for the lactate determination immediately. The third portion was ground with-

TABLE 5

The effect of concentration of iodoacetic acid upon lactate accumulation in macerated muscle

EXPERIMENT	TYPE AND WEIGHT OF MUSCLE	IDOACETIC INJECTED	INCUBATION PERIOD	LACTIC ACID POUND	
			hours	per cent	
28 {	1 Thigh, 3.61 gram	1/5,000	3	0.008	
28 {	2 Thigh, 3.67 gram		0	0.008	
19 {	1 Thigh, 1.78 gram	1/6,000	0	0.011	Macerated before freezing
19 {	2 Thigh, 1.66 gram		0	0.011	Frozen, then macerated
19 {	3 Thigh, 3.28 gram		2	0.014	
30 {	1 Thigh, 1.76 gram	1/15,000	3	0.015	
30 {	2 Thigh, 2.03 gram		0	0.012	Macerated before freezing
31 {	1 Thigh, 3.07 gram	1/30,000	3	0.230	
31 {	2 Thigh, 2.49 gram		0	0.128	Macerated before freezing
20 {	1 Thigh, 2.01 gram	Control	0	0.126	Macerated before freezing
20 {	2 Thigh, 2.52 gram		0	0.081	Frozen, then macerated
20 {	3 Thigh, 4.37 gram		2	0.460	

out freezing and then precipitated with tungstic acid, in order to induce the maximum lactic acid production possible through extensive injury (table 5).

High concentrations of iodoacetic acid inhibited lactate accumulation completely in all portions, even when minced prior to freezing, in great contrast to the normal muscle. Normal muscle taken as a control showed a high original level, as would be expected due to injury in dissection, a still higher level following grinding prior to freezing, and the usual high lactate accumulation following incubation. Thirty-three thousandths milligram of iodoacetic acid per gram of tissue only partially inhibited lactate accumulation, in complete accord with the results obtained in intact muscle. It is obvious that iodoacetic acid affects the production of lactic acid in minced muscle.

3. *Effect of iodoacetic acid on the rate of lactic acid production in intact muscle.* It seemed apparent to us that the final concentration of lactate in iodoacetic acid poisoned muscle following any experimental treatment depends upon the concentration of iodoacetic acid to which the muscle has been subjected. This fact suggested the fundamental importance of turning toward an investigation of the manner in which the carbohydrate-breakdown cycle is modified.

Evidence presented by the majority of the foregoing experiments pointed toward the conclusion that iodoacetic acid does not alter the rate of disappearance of lactic acid. Given equal numbers of stimuli and constant time intervals between beginning of stimulation and the immersion in liquid air, there occurs in normal muscle a high final lactic acid content, in lightly poisoned muscle a lower final content, and in heavily poisoned muscle a complete inhibition of lactic acid production. It is a matter of common observation that a delayed lactic acid production in normal muscle is difficult to detect by chemical means, regardless of the speed with which the control muscle is frozen following stimulation. This partial inhibition of lactic acid formation encountered at low concentrations therefore raised a further question. Does iodoacetic acid effect a limitation of the final concentration to which lactic acid may accumulate? This seemed unlikely. It seemed more probable that the rate of the reaction of which lactic acid is the end product is so retarded that at equal intervals of time following any disturbance which tends to shift the precursor-lactic acid equilibrium to the right, the reaction in poisoned muscle and in normal muscle can not have proceeded to the same extent.

Iodoacetic acid was injected in the usual manner, in two concentrations, 0.165 mgm. per gram, and 0.033 mgm. per gram, and dissection of the gastrocnemius muscles made after intervals of from 35 to 70 minutes. One muscle of each pair was fatigued and frozen immediately, the other was fatigued and placed for 20 minutes in a paraffin chamber through which nitrogen flowed constantly. Experiments 15 and 16, table 6, show a considerable lactate accumulation during the post-contraction interval, while normal muscles and muscles poisoned with higher concentration of the drug show no accumulation. The delayed lactate production, which may be termed the "recovery" lactate production in view of recent observations concerning the order of chemical events in the contraction process (Lippman and Meyerhof, 1930) and the function of lactic acid formation (Lundsgaard, 1930) indicates that the fundamental alteration in iodoacetic acid poisoned muscle is an inhibition of the rate of lactic acid formation. That this rate may become infinitely slow as the iodoacetic acid concentration increases is indicated by the complete suppression of lactate accumulation encountered above 0.05 mgm. of iodoacetic acid per gram.

4. *The influence of concentration of iodoacetic acid upon the rate of lactic*

acid production in minced muscle. The foregoing observation that the final lactate content in both minced and intact muscle is dependent upon the iodoacetic acid concentration and the conclusion that this is the resultant effect of an inhibition of the rate of lactic acid formation, led us to an attempt to study the uncomplicated effect of concentration of iodoacetic acid upon the rate of lactic acid formation.

Large bull frogs, *R. catesbiana*, were killed by transecting the spinal column anterior to the terminal lumbar vertebra, followed by a transverse

TABLE 6
Influence of iodoacetic acid upon rate of lactate production

EXPERIMENT	IDOACETIC INJECTED	TIME AFTER INJECTION	PROCEDURE	LACTIC ACID FOUND	
		<i>minutes</i>		<i>per cent</i>	
15 {A B}	1/30,000	35	Fatigued Fatigued	0.054 0.128	Frozen immediately In N ₂ 20 minutes after stimulation
16 {A B}	1/30,000	70	Fatigued Fatigued	0.031 0.123	Frozen immediately In N ₂ 20 minutes after stimulation
17 {A B}	1/6,000	40	Fatigued Fatigued	0.018 0.018	Frozen immediately In N ₂ 20 minutes
18 {A B}	1/6,000	55	Fatigued Fatigued	0.015 0.015	Frozen immediately Frozen immediately
19 {A B}	1/6,000	60	Non-irritable Non-irritable	0.012 0.013	Frozen immediately Frozen immediately
20 {A B}	Control		Fatigued Fatigued	0.242 0.256	Frozen immediately In N ₂ 20 minutes
21 {A B}	Control		Fatigued Fatigued	0.210 0.215	Frozen immediately In N ₂ 20 minutes

cut across the head at the angle of the mouth, and destruction of the spinal cord with a probe posteriorly from the latter cut. As much as possible of the total muscle of the hind legs was removed and frozen by immersion in liquid air after division into suitably small pieces with scissors. The frozen pieces were forced through a meat grinder, previously cooled with liquid air, and the resulting coarse particles ground with a pestle to a fine powder in a pre-cooled mortar. The powder was thoroughly mixed, covered to prevent condensation of water vapor upon it, and kept frozen by repeated applications of liquid air. A separate set of beakers was pre-

pared for each concentration of iodoacetic acid to be used, each beaker in the set corresponding to a definite incubation time. Into each beaker were placed 10 cc. of Ringer containing such an amount of iodoacetic acid that its final concentration following the addition of a pre-determined weight of muscle powder would be that desired. The beakers were then weighed, and covered to prevent evaporation. A set of beakers containing Ringer alone served as a control. Muscle powder was first roughly apportioned to each beaker by means of a cooled spatula which delivered the desired weight of tissue within satisfactory limits when uniformly filled. The beakers were weighed on a quick weighing quadrant balance, sensitive to

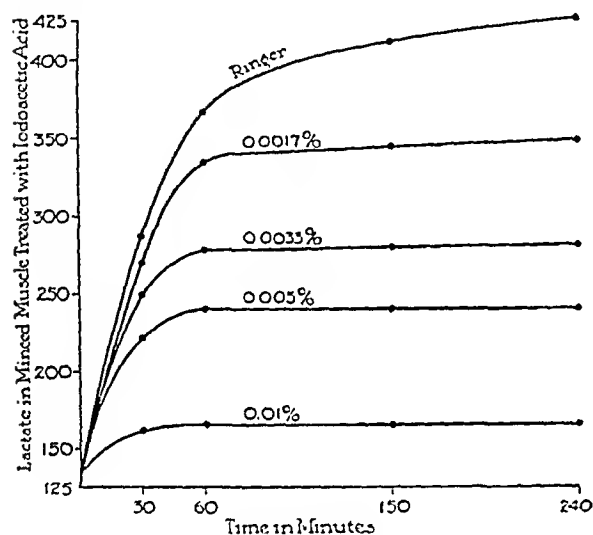


Fig. 1

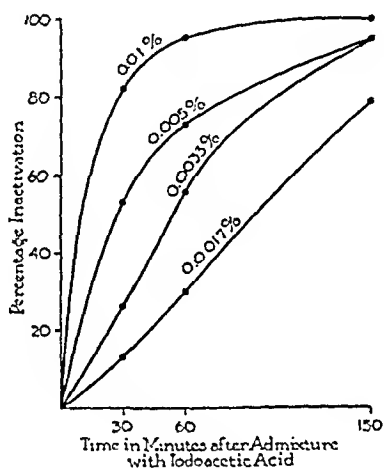


Fig. 2

Fig. 1. Lactic acid formation in minced muscle treated with the concentrations of iodoacetic acid noted on the separate curves in comparison with the rate of formation in Ringer's fluid.

Fig. 2. The rate of inactivation of the enzyme system by iodoacetic acid. The percentage inactivation of the enzyme system is determined over the course of time for several concentrations of iodoacetic acid.

2.5 mgm., to determine the exact weight of muscle used. The tissue mass was then dispersed with a stirring rod. One beaker from each set was taken at zero time, the proteins being precipitated as described above as quickly as possible after weighing. Similarly, one beaker from each set was taken at 30, 60, 150 and 240 minutes.

Except for the expected variations in the original lactate content, the results of 6 complete experiments agree closely. A composite graph of a typical experiment is shown in figure 1.

There was a uniformly approximately complete inhibition of lactic acid production by 0.01 per cent iodoacetic acid, and a graded inhibition by

lower concentrations, proceeding in a strikingly regular fashion. One point of similarity between all the experiments has led to a more critical examination of our results from a physico-chemical point of view.

It is to be noted that lactic acid formation in muscle powder in the presence of iodoacetic acid tends toward a maximum concentration peculiar to the particular percentage of iodoacetic acid. In no case does the final concentration of lactic acid in the presence of iodoacetic acid become equal to that in the presence of plain Ringer, even at the low concentration of 0.0017 per cent, over a period of 4 hours. Between 60 and 150 minutes, and between 150 and 240 appreciable lactic acid continues to be formed in muscle powder in Ringer, while the slope of the curves representing lactic acid formation in the presence of iodoacetic acid has uniformly approached zero.

If iodoacetic acid inhibits the action of glyoxalase, it appears to us that this inhibition must be effected in one of two ways. Iodoacetic acid might act as a retarding agent without itself entering into any chemical reaction with any component of the system, in the manner of a negative catalyst. This possibility is rendered unlikely by the observation that the inhibition of lactic acid formation in the case of all concentrations of iodoacetic acid progressively increases with time. It seems reasonable that if any lactic acid production is to be noted in the early minutes, it should proceed at that retarded rate, eventually to reach a concentration equal to that in the uninhibited system at infinite time. Our observations obviously do not fit in with this concept. If, on the other hand, the inhibition is effected by an inactivation of some component of the system, the observations recorded above are those which might be expected. If the action of iodoacetic acid were an inactivation of glyoxalase, this inactivation proceeding at a rate determined by the concentration of one of the reactants, in this case, iodoacetic acid, then a progressively increasing inhibition of lactic acid formation would be expected as a result.

That a progressive increase in inhibition does occur is shown by figure 2, where the rate of inactivation of the enzyme system is calculated and the average from three experiments is plotted against time. During the later intervals there is also a decrease in the rate of lactic acid formation in the Ringer control, as the lactic acid maximum is approached. The apparent reason for this progressive decrease in rate in Ringer is the gradual depletion of the substrate. Since it is a matter of common experience that lactic acid accumulation in muscle *brei* proceeds to depletion of carbohydrate stores only after the addition of phosphate, it seems to be an essential part of the substrate in such a system, and its concentration may in this instance be the limiting factor. It is obvious that a direct comparison of the increase of lactic acid in the iodoacetic acid Ringer and in the plain Ringer for corresponding time intervals is open to serious criticism. At

the beginning of any time interval except the first, since lactic acid production has not proceeded to the same extent in both Ringer and iodoacetic acid Ringer, the concentration of substrate likewise cannot be identical, and the true inhibition by iodoacetic acid is partially masked by the "substrate depletion" inhibition of the control system.

In order to calculate the inhibition of the enzyme system due to iodoacetic acid, it is necessary to measure the extent of inhibition not at identical times in the poisoned and the unpoisoned system, but at identical concentrations of the substrate reactants. Thus in figure 1, for example, in determining the percentage inhibition of the system with 0.0033 per cent iodoacetic acid at 60 minutes' time, one cannot compare the rate during the next hour with the rate of the control experiment during that same hour, because in the latter nearly twice as much of the substrate had already been used up at that point. It is therefore necessary to compare the slope of the lactic acid formation curve in the iodoacetic treated muscle at 60 minutes with the slope of the curve for the untreated muscle at a corresponding concentration of substrate reactants, which happens to have existed in this experiment at approximately 28 minutes after the onset of the reaction. This calculation depends upon the assumption that the lactic acid formed is a reliable measure of the amount of original reactant used up. This assumption is undoubtedly permissible unless there is an important intermediary product between the reaction influenced by iodoacetic acid and the lactic acid. This seems extremely unlikely, because iodoacetic acid is known to inhibit glyoxalase which is itself the enzyme responsible for the last step in the formation of lactic acid.

The rate of lactic acid formation in the iodoacetic system is expressed as the per cent of the rate under similar conditions in the normal system. This figure is subtracted from 100 in order to express the results as percentage inactivation. It is apparent from figure 2 that the rate of inactivation is a function of the concentration of iodoacetic acid and that it tends towards complete inactivation at all concentrations, although in the lowest concentrations used the inactivation is not rapid enough to be completed before a large share of the substrate has been acted upon by the active enzyme system.

If one plots the logarithm of the enzyme concentration against time during the inactivation by iodoacetic acid, one finds a linear relation between the two which must be interpreted as indicating that in the kinetics of the process at a given concentration of iodoacetic acid the concentration of the enzyme itself limits the rate of its inactivation. Thus it appears to be a reaction of the first order, although obviously it is not monomolecular. It seems likely that the iodoacetic acid itself does not enter into permanent combination with the enzyme.

Lohmann (1931) in a study on hexose-phosphoric acid esterification in

muscle extract has called attention to the fact that the action of mono-iodoacetic acid differs from that of fluoride. Whereas poisoning by the latter is practically instantaneous, poisoning by mono-iodoacetic acid is a "time reaction," requiring as long an interval as 60 minutes for maximum ester accumulation. He postulates, in the case of mono-iodoacetic acid, an irreversible alteration of the protein component of the enzyme system. Meyerhof and Boyland (1931) have also established that poisoning of frog sartorii by immersion in mono-iodoacetic acid is a process involving considerable time, which they interpret as being determined by the rate of diffusion into the interior of the muscle. It seems that in our experiments with powdered muscle diffusion rate does not play a rôle after the first few minutes.

CONCLUSIONS

1. Preformed or added lactic acid does not disappear during anaerobic recovery in iodoacetic acid poisoned muscles.

2. Muscles poisoned with low concentrations of iodoacetic acid show only partial inhibition of lactic acid production as a result of stimulation. Iodoacetic acid depresses the irritability of muscles but the inhibition of lactate formation is not due to this non-irritability, since chloroform rigor fails to produce lactic acid in poisoned muscles.

3. With low concentrations of iodoacetic acid there is in contrast to the situation in normal muscles and in those treated with high concentrations of iodoacetic a large delayed lactic acid production following stimulation.

4. The rate of lactate formation in minced muscle is inhibited by iodoacetic acid in direct proportion to the concentration of the poison. The inhibition increases progressively, indicating an inactivation proceeding with measurable velocity.

BIBLIOGRAPHY

- LUNDGAARD, E. 1930. *Biochem. Zeitschr.*, ccxvii, 162, 51.
DUDLEY, H. W. 1931. *Biochem. Journ.*, xxv, 439.
CASE, E. M. AND R. P. COOK. 1931. *Biochem. Journ.*, xxv, 1319.
VISSCHER, M. B. AND P. W. SMITH. 1930. *This Journal*, xcv, 121.
FRIEDEMANN, T. E., M. COTONIO AND P. A. SHAFFER. 1927. *Journ. Biol. Chem.*, lxxiii, 335.
LIPMANN, F. AND O. MEYERHOF. 1930. *Biochem. Zeitschr.*, ccxxvii, 84.
LOHMANN, K. 1931. *Biochem. Zeitschr.*, ccxxxvi, 444.
MEYERHOF, O., AND E. BOYLAND. 1931. *Biochem. Zeitschr.*, ccxxxvii, 406.

THE RESPIRATORY METABOLISM OF PANCREATIC DIABETES IN CATS

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In pancreatic diabetes, Falta, Grote and Staehelin (1907) showed that the dog's metabolism was increased by 42 per cent. Murlin and Kramer (1913) obtained a similar result with their dog. Enderlen, Glatzel and Pú (1928) found an increase above the basal metabolism ranging between 3 and 28 per cent. Hédon (1927) in his pancreatectomies left a remnant of the tail just under the skin to supply the pancreatic hormone. When the animal had fully recovered from the laparotomy, then the graft was removed and the metabolism measured. Eight out of eleven dogs showed an increased metabolism. One diabetic animal to which insulin was given showed a normal metabolism which increased when the insulin was withdrawn.

In human diabetes Benedict and Joslin (1912) found an increase of about 20 per cent. Allen and DuBois (1916) concluded from their own results and a reevaluation of those obtained by Magnus-Levy, Mohr, and Benedict and Joslin, that although some results were high the average metabolism of diabetes showed no marked increase. Joslin (1923) states concerning his work that "23 per cent of the total number of observations, representing 27 per cent of the patients for whom the Harris-Benedict standard was used in the post-absorptive state, varied more than 20 per cent above or below the center of the normal zone of metabolism."

It seems surprising that the dog should always show an increased metabolism in diabetes and the results on man be so variable. Perhaps the explanation lies in the fact that in human diabetes the islet cells are almost never entirely destroyed and therefore the disease is not as complete as after extirpation. At least, under the circumstances, it appeared worth while to study the metabolism of diabetes in another animal. This was done in cats.

METHOD. The open-circuit method of measuring metabolism was used. Compressed air under a constant pressure was passed through the animal chamber at a rate such that the outgoing air contained about 1 per cent CO₂. The animals were kept comfortable by having the lower half of the respiration chamber in a water bath at 30°C. and by warming the ingoing

air to the same temperature. Taking these precautions, we found that our cats would become quiet very quickly and would often remain so for hours. A graphic record of activity told us when the animal was still, and at half-hour intervals thereafter we sampled the outgoing air and later analyzed it for carbon dioxide and oxygen. Usually two out of three metabolism measurements by this method checked satisfactorily for our purposes.

The analyses of the samples were made in three gas analyzers of the 10 cc. Carpenter type. Results on the same sample in different analyzers were checked within 0.04 per cent.

No estimations of urinary nitrogen were made. The metabolism calculations are based on carbohydrate and fat combustion alone, which of course makes all the figures too high. It is possible that the error involved in not measuring urinary nitrogen is more serious in the diabetic than in the normal animals. Yet if we assume that these cats burn only protein and that 58 per cent of this is converted to sugar and excreted, our calculations would not have to be lowered by more than 13 per cent. This would still leave a marked difference between the metabolism of the normal and the diabetic cat.

The Meeh formula as given by Lusk (1928) was used in the estimations of surface area. The results are given in calories per square meter per hour.

In most of our cats, the metabolism was measured a few days prior to pancreatectomy. After the removal of the pancreas, urine sugar and acetone were qualitatively determined by Benedict's and Rothera's tests respectively. Usually about four units of insulin were given on the second day after the operation and this amount was increased until the urine became acetone-free. It was impossible for us to regulate the dosage of insulin to make the urine free from sugar without at the same time producing hypoglycemia and convulsions.

RESULTS. Cats differ greatly from dogs in their responses to diabetes. For example, Soskin (1930) has had a diabetic dog on a protein diet that survived thirty-three days after the withdrawal of insulin. In this animal the acetone in the urine gradually rose to a maximum on the fourth day and then diminished. In some dogs the acetone actually disappeared from the urine.

Cats on a diet of salmon, pancreas and milk usually show acetone in the urine by the second day after stopping insulin and may even die then, though they usually survive until the fourth day when the acidosis becomes very severe. At this time insulin treatment is almost always ineffective in bringing about recovery. Acidosis is less likely to appear if a high carbohydrate diet is given but the survival period is not changed.

The metabolism of our cats was determined before the operation and

then at least a week after recovery. The results of 41 observations on 19 normal cats showed an average metabolism of 29.3 ± 0.4 calories per square

TABLE 1

CAT	DATE		WEIGHT	RESPIRA- TORY QUOTIENT	CALORIES PER SQUARE METER PER HOUR
			<i>kgm.</i>		
514	Dec. 16		2.8	0.85	30.0
					30.7
	Jan. 4	Depancreatized			
	Jan. 13	2nd day without insulin	2.0	0.70	50.6
				0.67	48.3
				0.67	48.0
	Jan. 14	3rd day without insulin	2.0	0.69	39.2
				0.73	39.0
	Jan. 20	With insulin	2.0	0.75	40.5
	Jan. 29	With insulin	1.6	0.67	43.3
516				0.71	42.5
	Dec. 17		3.7	0.78	32.8
				0.71?	32.1
	Dec. 21	(Partial thyroidectomy)			
	Dec. 29		3.1	0.78	31.6
				0.77	30.7
	Jan. 4	Depancreatized			
	Jan. 11	With insulin	2.8	0.75	41.8
				0.75	41.8
	Jan. 14	With insulin	2.7	0.65	40.1
991				0.70	40.2
	Jan. 21	With insulin	2.6	0.76	40.1
				0.70	39.4
	Sept. 18			0.82	31.5
	Sept. 22		2.7	0.85	31.6
	Sept. 23	Depancreatized			
	Oct. 1	Insulin 24 hours before	2.2	0.74	39.0
				0.71	39.8
	Oct. 3	Insulin 24 hours before	2.1	0.76	39.9
				0.77	38.4
	Oct. 7	2nd day without insulin	2.0	0.68	42.2
				0.71	38.5
	Oct. 14	Insulin 24 hours before	1.8	0.81	43.3
				0.83	43.2

meter per hour, the maximum single observation being 34.8 calories. The respiratory quotients for the series averaged 0.75 ± 0.006 .

With one exception, these cats, made diabetic, showed a high metabolism on the second day without insulin. The mean of 28 observations on 10 cats

that survived for two weeks or more is 41.7 ± 0.4 , the lowest result being 36.6 calories. The respiratory quotients averaged 0.70 ± 0.004 . The increase for the series was 42 per cent. The smallest rise for any animal was 13 per cent. The constancy of the increased metabolism is very surprising in view of the marked inanition shown by some (see table 1). This is in contrast to the results obtained by Allen and DuBois (1916) and Joslin (1923) on human diabetic subjects undergoing the Allen treatment in which under-nutrition was common and the average metabolism fell below normal.

The one exception which we have not included in the series above was No. 129 shown in table 2. This cat had a 13 per cent increase in metabolism on the second day without insulin, but its heat production in diabetes was

TABLE 2

CAT	DATE		WEIGHT	RESPIRATORY QUOTIENT	CALORIES PER SQUARE METER PER HOUR
			<i>kgm.</i>		
129	Jan. 5	Depancreatized			
	Jan. 9	With insulin	2.3	0.71	29.2
				0.72	29.3
				0.71	29.7
	Jan. 11	2nd day without insulin	2.2	0.72	31.0
				0.66	34.9
				0.65	33.5
	Jan. 12	3rd day without insulin	2.1	0.70	34.8
				0.71	33.1
	Jan. 13	4th day without insulin	2.0	0.69	47.9
				0.68	48.5
				0.73	46.2

so close to the results for normal cats that it seemed possible that the pancreas had not been completely removed. Yet, on the fourth day without insulin, the metabolism jumped to a very high level and the animal died in severe acidosis in spite of receiving insulin.

We expected that all diabetic cats would show a normal metabolism when given insulin. This, however, was not true. Varying the dosage of insulin or the time interval between the injection and the metabolism measurement failed in a number of cats to bring the heat production down to normal.

A few animals, for unknown reasons, did show normal metabolism when given insulin after pancreatectomy. The results on these animals are given in table 3. It will be noted that the second day after the withdrawal of insulin the metabolism had risen to the diabetic level.

The removal of the head of the pancreas, leaving the tail to supply

insulin, brought about no change in metabolism. In 25 observations on such cats, the respiratory quotients averaged 0.75 ± 0.001 and the

TABLE 3

CAT	DATE		WEIGHT	RESPIRA- TORY QUOTIENT	CALORIES PER SQUARE METER PER HOUR
			<i>kgm.</i>		
191	Sept. 22		1.9	0.80	27.3
	Sept. 25	Depanereatized			
	Oct. 6	Given insulin	?	0.76	29.3
	Oct. 8	Given insulin and glucose	1.9	0.93	30.3
	Oct. 13	2nd day without insulin	1.7	0.70	41.5
	Oct. 15	2nd day without insulin	1.5	0.69	41.0
521	Dec. 22	Head of pancreas removed			
	Jan. 7		2.5	0.80	29.1
					30.7
	Jan. 12	Rest of pancreas removed			
	Jan. 20	2nd day without insulin	2.3	0.74	39.4
				0.69	42.7
				0.68	43.9
	Jan. 22	With insulin	2.3	0.72	
532				0.69	31.0
				0.68	32.4
	Jan. 18	Depancreatized			
	Jan. 26	1st day without insulin	2.9	0.71	30.3
				0.74	29.1
	Jan. 27	2nd day without insulin	2.8	0.76	38.6
534				0.74	40.2
				0.74	38.5
	Jan. 28	Depanereatized			
	Feb. 6	4 units insulin 24 hours before	2.3	0.68	39.5
				0.70	38.8
	Feb. 10	10 units insulin 24 hours before	2.1	0.74	32.5
992					
	Feb. 13	No insulin for 48 hours	1.9	0.74	43.6
				0.71	46.6
	Sept. 23		2.5	0.82	29.1
	Sept. 28	Depanereatized			
	Oct. 6	Insulin 24 hours before	2.2	0.74	29.1
				?	30.0
	Oct. 8	Insulin and glucose 24 hours before	1.9	0.70	42.4
				0.70	41.1

metabolism per square meter per hour was 29.4 ± 0.6 —the same as un-operated cats. Table 4 shows observations on such a cat.

We are now investigating the reason for the rise in metabolism after complete pancreatectomy.

TABLE 4

CAT	DATE		WEIGHT	RESPIRATORY QUOTIENT	CALORIES PER SQUARE METER PER HOUR
503	Nov. 16	Partial pancreatectomy	kgm.		
			2.35	0.83	31.0
	2.35		0.80	32.6	
	Nov. 17		0.83	30.8	
	Nov. 30		2.0	0.70	31.8
	Dec. 4			0.75	28.1
				0.75	28.0
	Dec. 7		1.9	0.77	28.9
				0.80	32.5

CONCLUSIONS

1. Normal cats in post-absorptive condition have an average metabolism of 29.3 ± 0.4 calories per square meter per hour and a respiratory quotient of 0.75 ± 0.006 (see tables 1 and 3).

2. If the head of the pancreas is removed, cutting off the supply of pancreatic juice to the intestine, the metabolism remains unchanged. The averages for these animals are 0.75 ± 0.001 for the respiratory quotient and 29.4 ± 0.6 for the metabolism (see table 4).

3. Complete removal of the pancreas lowers the respiratory quotient to 0.70 ± 0.004 and increases the metabolism more than 40 per cent to 41.7 ± 0.4 calories (see tables 1, 2 and 3). This occurs even in under-nutrition.

This work was done at the suggestion of Dr. W. B. Cannon.

BIBLIOGRAPHY

- ALLEN, F. M. AND E. F. DuBOIS. 1916. Arch. Int. Med., xvii, 1010.
 BENEDICT, F. G. AND E. P. JOSLIN. 1912. Carnegie Inst. Washington, Pub. 176.
 ENDERLEN, E. H., H. GLATZEL AND PÚ. 1928. Arch. f. exp. Path. u. Pharm., cxxxix, 20.
 FALTA, W., F. GROTE AND R. STAEBELIN. 1907. Hofmeister's Beitr. chem. Physiol. u. Path., x, 199.
 HÉDON, L. 1927. Arch. internat. de Physiol., xxix, 175.
 JOSLIN, E. P. 1923. Carnegie Inst. Washington, Pub. 323.
 LUSK, G. 1928. Science of nutrition. 4th ed., p. 122, W. B. Saunders Company.
 MURLIN, J. R. AND B. KRAMER. 1913. Journ. Biol. Chem., xv, 380.
 SOSKIN, S. 1930. Journ. Nutrition, iii, 106.

THE PLACE OF THE RED NUCLEUS IN THE POSTURAL COMPLEX

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It has been believed in various quarters that the differences in behavior between decerebrate animals of the Sherrington type and those in which the transection has been made through or in front of the thalamus depends upon the structural and functional integrity of the red nucleus in the latter instances. The experiments of Rademaker (1926) have tended to support this assumption, the work of this investigator indicating that section of the decussation of Forel or destruction of the red nuclei by gross lesions induces a condition of greatly increased extensor tonus with loss of labyrinthine and "body on the body" righting reflexes, and renders the animal practically helpless. It has appeared not unlikely, however, that Rademaker's conclusions were based upon surgical procedures which may have involved other important centers in the brain stem, and it has been suggested that the radical effects produced were due to injury of certain portions of the vestibular complex as well as of the red nucleus. The position of the latter, deeply situated as it is, precludes the use of ordinary techniques in attempting its ablation. Lesions produced in the conventional manner are almost sure to damage so great a mass of tissue as to detract from the reliability of the results.

The present writers have recently reported a series of observations on cats in which the greater portions of the red nuclei were destroyed by the use of the Horsley-Clarke stereotaxic instrument (Ingram and Ranson, 1932). This apparatus, the use of which has been discussed in earlier publications (Ingram, Ranson, Hannett, Zeiss and Terwilliger, 1932; Ingram, Ranson and Hannett, 1932), enables one to place small electrolytic lesions in the interior of the brain with a minimum of damage to regions outside the structure which it is wished to destroy. Following the destruction of that portion of the red nucleus caudal to the middle or rostral fibers of the third nerve, it was found that the cats retained their ability to right themselves, stand and walk to a degree which enabled them to live normal lives. According to Rademaker, such lesions eliminate the labyrinthine and "body on the body" righting reflexes, leaving the optic, cervical and "body on the head" reflexes intact. If this is so, the two latter must have

been sufficient in our cases, since after bilateral enucleation of the eyes the animals were still able to get on their feet and walk. Increase in tonus of the extensor muscles was also observed in this group of cats. These tonus changes were of mild character, insufficient to interfere with locomotion, and were noticeable only under suitable conditions. There were also certain abnormalities of the gait, such as dysmetria, poor coördination, and a slight degree of ataxia.

In this series of experiments the greater portion of each red nucleus, or its descending connections, was destroyed, but in practically every instance a small part of the rostral, diffuse portion of the nucleus was left, rostral to the third nerve. Since, according to Mussen (1927), lesions in this latter region cause symptoms which are quite different from those following damage to the pars compacta, and since it was desirable to further check and extend previous observations, another group of operations was performed in attempting to eliminate the red nucleus in its entirety. The results of these efforts are reported here.

METHODS. The Horsley-Clarke stereotaxic instrument makes possible the introduction of a bipolar needle electrode to any point within the brain, whose location has been determined previously in terms of the orientation planes of the instrument. Passage of a direct current between the poles of the electrode produces, at this point, an electrolytic lesion the extent of which depends upon the amount and duration of the current used. The electrodes are made of nichrome wires cemented together and reinsulated with chlorinated rubber solution. The bare tips of the wires are less than one millimeter apart, making possible the confinement of the influences of the current to a restricted area. For total destruction of the red nucleus a row of small lesions, one millimeter apart, was produced in the latter over a total extent of five millimeters rostrocaudally. A current of about one milli-ampère and of thirty to sixty seconds' duration was used. Lesions so placed fuse together to form a single elongate lesion affecting the nucleus ruber or its connections without serious damage to other portions of the brain stem. In many instances the red nuclei were directly destroyed, in others the lesions were placed so that the rubro-spinal tracts or the decussation of Forel were interrupted, with the nuclei themselves but partially involved. In the cases to be described the rubro-spinal and rubro-reticular tracts were completely interrupted either at their sources in the red nucleus, in the decussation of Forel, or in the proximal part of their course after crossing.

These experiments were all of the so-called acute type; no aseptic precautions were taken and the animals were killed within twenty-four hours after operation. The lesions were produced under ether anesthesia. Observations of the post-operative behavior of the animals were made after sufficient time for recovery from the anesthetic had elapsed. The ability of each animal to right itself, stand and walk was carefully studied, as was

the state of tonus of the extensor muscles. Changes in the degree of tonus were readily observed when the animal was suspended in a sort of canvas hammock with the legs protruding through holes. Normal animals in this situation ordinarily hold the limbs flexed and offer no consistent resistance to passive flexion of the legs although variable voluntary resistance is often encountered, but if there is any extensor hypertonus the limbs are held more or less extended and moderately stiff. There is under such circumstances a certain amount of resistance to passive flexion induced by grasping the leg above the wrist or ankle and moving it forcibly back and forth. The rigidity is more regularly elicited when the upward pressure is exerted against the pads of the toes in the position for the positive stütz reflex. With practice, this procedure offers valuable means of estimating the degree of slight changes in extensor tonus.

At the conclusion of the experiment the animal was killed, and the brain fixed by injecting the head with a solution of formaldehyde, U.S.P., 1:10. The brain stem was removed, imbedded in celloidin, and cut into fifty micron sections, every fourth one of which was stained for myelinated fibers by Weil's method, or with cresyl violet for cells, and mounted. Outline drawings of certain of these sections were made with the aid of the Edinger drawing apparatus to facilitate the determination of the extent of the lesions and the structures involved.

OBSERVATIONS. The cases to be discussed include seven cats in which the red nuclei or their descending connections were completely destroyed. Five of these were able to right themselves, stand and walk, even when the visual righting reflexes had been eliminated by blindfolding or enucleation of the eyes. Of the other two, one could not stand or walk; the other could stand, could right itself to some extent, but did not walk. Those able to progress will be discussed first and a brief description of each animal's behavior given. The accompanying figures illustrate the extent of the lesion in one of these cases, cat 231, for which a more complete protocol will be included.

Cat 231 suffered elimination of the optic righting reflexes by enucleation. Following production of the lesions involving the red nuclei and recovery from the anesthetic it was able to right itself from either side when placed on the ground. It walked with a poorly coordinated gait, extending the forelimbs far forward, while the hindlimbs tended to shoot back or to the side. Frequently the feet were poorly placed, so that the weight was thrown upon the dorsum of the wrist or upon the subverted toes of the hind feet. There was a tendency to turn in a circle to the right. When suspended in the hammock this animal was able to flex all four limbs and hold them flexed voluntarily, but when at rest all except the left hindlimb were extended. There was a marked stütz reaction in all except the left hindlimb, in which it was moderate. Moderate resistance to passive flexion was observed in all but the left hindlimb, in which it was slight.

The extent of the lesions in this case is illustrated in figures 1 to 6. The first two figures show the rostral poles of the red nuclei to have been completely destroyed, the lesions also involving the rostral portions of the rubro-spinal tracts; the section shown in figure 2 was taken 0.7 mm. caudal to that in figure 1. Figure 3 was taken from a level approximately 0.5 mm. caudal to figure 2; figure 4, 0.65 mm. caudal to figure 3; figure 5, 0.65 mm. caudal to figure 4; and figure 6, 0.1 mm. caudal to figure 5. The last four figures show that while the medial portion of the left and lateral portion of the right red nucleus escaped destruction, the rubro-spinal tracts were completely destroyed. The lesions were confluent and the zone of injury continuous rostro-caudally. Structures outside the red nucleus and rubro-spinal tract were not seriously damaged, although the right nucleus interstitialis was somewhat affected by the needle puncture, a fact which probably accounts for the tendency of the animal to circle to the right. Fibers of the brachium conjunctivum were also involved, of course, as they passed through the red nucleus.

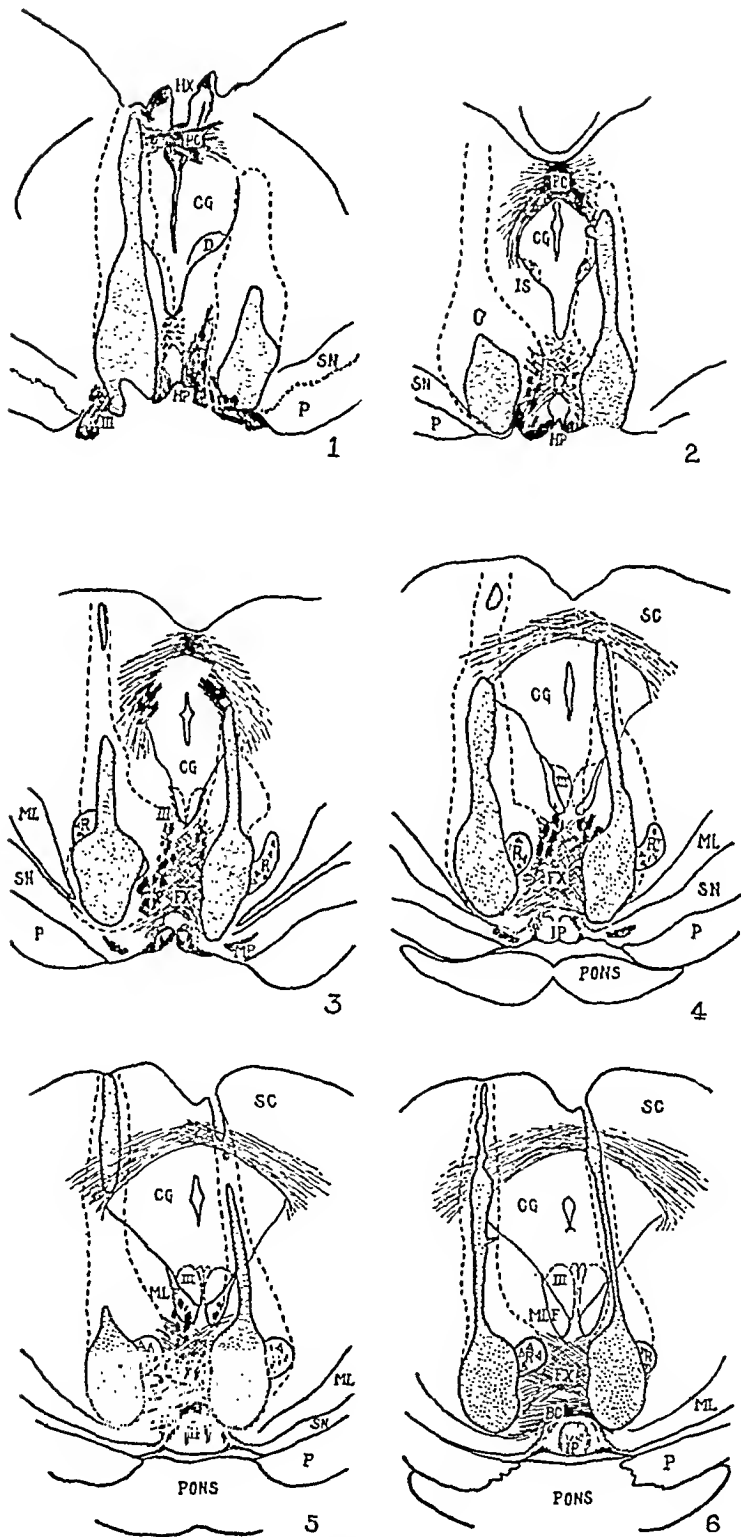
In cat 229 the eyes were enucleated at the time the red nuclei were destroyed. Five hours after the operation this animal was able to right itself from either side, stand, and walk. The gait was awkward and ataxic, and there was pronounced scissoring of the forelimbs. There was a tendency to circle to the right. In the hammock rather marked rigidity was observed, there being a good stütz reaction in each limb, and good resistance to passive flexion in all but the left hindlimb, in which it was slight.

In this case the lesion involved the medial portions of both red nuclei and destroyed the rubro-spinal tracts throughout their course in the mesencephalon. The red nuclei were involved in the edematous zone of the lesion. The interstitial nuclei were partially injured, as was the lateral part of the right medial longitudinal fasciculus. The lesions extended from the caudal pole of the mammillary nucleus to the pons.

Cat 233 was also subjected to enucleation of the eyes at the time of operation. Six hours later it was able to right itself and stand, and, although apathetic and disinclined to activity, walked with an unsteady, ataxic gait. The extensor muscles showed considerable rigidity when the animal was placed in the hammock, the stütz reaction being readily obtainable in each limb. There was fair resistance to passive flexion in the forelimbs, but slight in the hindlimbs.

The lesions in this instance extended from the region just caudal to the mammillary bodies to the pons. The red nuclei were practically completely destroyed and the rubro-spinal tracts interrupted. There was some damage to the left nucleus of Darkschewitsch and nucleus interstitialis.

Cat 235 was able to right itself, even when blindfolded by a mask which covered the head, and could stand. It walked awkwardly, circling to the left, and exhibited considerable dysmetria and ataxia. When suspended,



Figs. 1-6

the forelimbs were extended, the hindlimbs partially so, and a good stütz reaction could be elicited in each leg. There was strong resistance to passive flexion in all but the right hindlimb, where it was slight.

In this instance the lesions also extended from the mammillary bodies to the pons, destroying the medial portion of the left red nucleus, all of the left rubro-spinal tract, and the portion of the decussation to the left of the midline. The lateral portions of the right red nucleus and rubro-spinal tract were destroyed; fibers in the latter, coming from the left nucleus ruber, were of course cut off more proximally by the lesion in the latter. Fibers from the medial portion of the right red nucleus were destroyed in the left rubro-spinal tract. The left medial longitudinal fasciculus was damaged at the level of the oculomotor nucleus, and the left nucleus interstitialis severely affected.

Cat 237 could right itself perfectly when not blindfolded. When masked it was able to get up from the right side, but manifested considerable difficulty in doing so when the left side was down. It stood in awkward postures, and in walking exhibited the incoördination characteristic of the other case. The extensor rigidity observed when the animal was suspended was quite marked; there was a good stütz reaction from each limb, and resistance to passive flexion, although slight, was present.

The lesions extended rostrally from the pons almost to the mammillary bodies, completely destroying the rubro-spinal tracts and inflicting con-

Fig. 1. Portion of transverse section through the brain stem of cat 231, showing the extent of lesions. The stippled area indicates the lesion; the area included within the broken line showed some slight evidence of edema.

Fig. 2. Portion of transverse section through brain stem of cat 231, taken approximately 0.7 mm. caudal to that in figure 1.

Fig. 3. Portion of transverse section through the brain stem of cat 231, taken approximately 0.5 mm. caudal to that in figure 2.

Fig. 4. Portion of transverse section through the brain stem of cat 231, taken approximately 0.65 mm. caudal to that in figure 3.

Fig. 5. Portion of transverse section through the brain stem of cat 231, taken approximately 0.65 mm. caudal to that in figure 4.

Fig. 6. Portion of transverse section through the brain stem of cat 231, taken approximately 0.1 mm. caudal to that in figure 5.

Abbreviations used in figures

<i>BC</i> , Brachium conjunctivum	<i>MLF</i> , Medial longitudinal fasciculus
<i>CG</i> , Central gray	<i>MP</i> , Mammillary peduncle
<i>D</i> , Nucleus of Darkschewitsch	<i>P</i> , Cerebral peduncle
<i>FX</i> , Decussation of Forel	<i>PC</i> , Posterior commissure
<i>HP</i> , Habenulo-peduncular tract	<i>R</i> , Red nucleus
<i>HX</i> , Habenular commissure	<i>SC</i> , Superior colliculus
<i>IP</i> , Interpeduncular nucleus	<i>SN</i> , Substantia nigra
<i>IS</i> , Interstitial nucleus	<i>III</i> , Third nerve or its nucleus
<i>ML</i> , Medial lemniscus	

siderable damage upon the red nuclei themselves. There was probably some injury to the right interstitial nucleus and to the nucleus of Darkschewitsch of the same side.

The two cats which did not walk perhaps deserve brief consideration. One of these, cat 230, was very inert and apathetic following the operation, which included enucleation of the eyes, lying on its side and making no attempt to rise, although it was capable of bringing its head to an upright position. When placed on its feet it could stand for a short time, but could not walk without falling forward with the wrists bent under and the hindlimbs extended. When suspended in the hammock some running movements and occasional generalized convulsive twitchings were observed. There was considerable rigidity, as manifested by high grade stütz reactions and resistance to passive flexion in all the limbs. The lesions in this cat were very large, meeting in the midline, and affecting the area just caudal to the mammillary bodies, the brachium conjunctivum on each side, the habenulo-peduncular tract, a large part of the central gray, the central tegmental fasciculi and the interstitial nuclei as well as the left nucleus of Darkschewitsch. The left red nucleus was damaged in its dorsal portion; the right red nucleus and rubro-spinal tract were entirely destroyed. All of the left as well as most of the right medial longitudinal fasciculus was destroyed.

Cat 232, although unable to walk when first examined, showed considerable improvement in the course of a few hours, and at the time it was killed there were indications that ability to walk would return shortly. However, during the time allotted for the experiment, attempts at walking were unsuccessful, although the animal could stand when placed on its feet. When lying on the floor the head was raised and occasional scratching movements were made as if in an attempt to raise the body. There were signs of forced movement which twisted the head so the right ear was lower than the left. When in the hammock there was marked extension of the limbs, with a good stütz reaction all around, and resistance to passive flexion which was marked in the forelimbs and moderate in the hindlimbs. This cat had also been subjected to enucleation, and it may well be that its failure to move about was due, in part at least, to inability to withstand the shock of the two operations. It is not unusual to find in a group, one or two animals whose stamina is not up to the average. The lesions were rather extensive, destroying the floor of the brain at the points where the habenulo-peduncular tracts reach their most ventral level, and causing general damage to the area just caudal to the mammillary bodies. The central gray, nucleus of Darkschewitsch and nucleus interstitialis on the left were largely destroyed. Both red nuclei were severely affected and the rubro-spinal tracts completely destroyed.

DISCUSSION. It is evident from the results presented here that the

behavior of animals in which the efferent descending connections of the red nuclei have been completely obliterated is not essentially different from that of animals deprived of the part of the red nucleus caudal to the third nerve, as described in a previous paper. Again we find a characteristically peculiar gait, mild extensor hypertonus and retention of ability to assume an upright position. Points at which these observations differ from those of Rademaker have been previously pointed out, but we can offer some corroboration of his work in regard to the increased tonicity of the extensor muscles. The rigidity observed by us is apparently of lower grade than that displayed by Rademaker's animals, a fact which may be in part accounted for by the integrity of the cerebral cortex in our cats. However, Rademaker has pointed out that mechanical destruction of the red nuclei in animals retaining the cortex gives rise to rigidity which is moderate compared with that obtained in decerebrate preparations.

Mussen has reported that an electrolytic lesion in the rostral pole of one red nucleus causes loss of the righting reflexes and a transient decrease in muscle tonus. The complete destruction of both red nuclei in our experiments did not produce these results.

The only way in which we can account for these discrepancies is by offering the suggestion that the amount of tissue involved by the lesions produced by these workers must have been greater than was realized at the time. Spitzer (1924) and Lorente de Nó (1928) believe that the rigidity occurring in Rademaker's cats was due to the cutting of a commissural path which passes between the two vestibular complexes and which ordinarily carries impulses inhibiting extensor tonus. It may be, also, that the loss of ability to stand and walk was due to injury to structures situated farther rostrally. In this connection it is interesting to consider our cat 230, above described, which exhibited no righting or walking reactions, and in which the rubral connections were apparently completely destroyed. Other cats, however, of which the latter was equally true were quite capable of orientation and progression, so we must look elsewhere than the red nucleus for the causes of the debility of cat 230. This animal possessed lesions which were much more extensive than usual, destroying a great portion of the medial part of the tegmentum. We also find involvement of the nuclei associated with the upper end of the medial longitudinal fasciculus and of the latter itself, which is perhaps suggestive. Cats showing partial involvement of these structures may show defects as to progression and righting which are typified by what are commonly called forced movements. In all nine of our animals which have shown tendencies to forced curvature to right or left, autopsy material has indicated that there was injury to either the medial longitudinal fasciculus, the nucleus of Darkschewitsch or the nucleus interstitialis on the side to which the forced movement occurred. May it not be possible that almost total or symmetrical

destruction of these structures will eliminate righting and walking capabilities?

We have been unable to determine satisfactorily the extent of any impairment to the righting reflexes in our walking cats, nor which of these reflexes are responsible for the existing righting reactions. That the optic mechanism does not participate is obvious, but we have not been able to decide whether or not the labyrinthine reflexes are ruled out in animals where the lesions are confined to the red nuclei. Observations of the righting reactions when the cats are held in the air by grasping the pelvis, as suggested by Magnus and Rademaker, have not given satisfactory and consistent results in our hands, perhaps because of the temperamental or constitutional responses of cats to such handling. In any case, these animals are generally able to orient themselves in an efficient manner, and it is certain that righting reflexes are not abolished in blind cats by the destruction of the red nucleus.

That the red nucleus plays a part in the regulation of tonus is indicated by our experiments. However, one must not overlook the possibility of such lesions as herein described involving the connections of the region immediately rostral to the red nucleus in the subthalamus, and we cannot be sure that this may not have been a factor in our results. It is, nevertheless, certain that bilateral destruction of the red nucleus does not produce a rigidity at all comparable to that seen in decerebrate animals, nor sufficient hypertonus to prevent locomotion.

The peculiarities of the gait, typified by dysmetria, poor coördination and ataxia, greatly resemble the effects of cerebellar injury. Lesions such as those necessary for destruction of the red nucleus necessarily involve the fibers of brachium conjunctivum, thus cutting off the best known efferent pathway from the cerebellum. If the rubro-spinal tract carried cerebellar influences caudalward one would also look for such symptoms as have been described here after its destruction. The so-called inferior syndrome of the red nucleus, described in a number of clinical cases, includes similar locomotor disturbances. While the nucleus ruber seems to participate in the regulation of tonus, its classical designation as a center for transmission and selection of cerebellar, cortical and pallidal influences acting on the final common pathway may still be regarded as eminently reasonable.

SUMMARY

1. Complete destruction of the red nuclei or their descending connections in the cat by restricted lesions leaves an animal capable of righting itself, standing, and walking, even after optic influences have been removed. It is therefore evident that the red nucleus cannot be regarded as an absolutely essential center for the righting function in blind animals.

2. The gait of such a preparation is usually characterized by dysmetria,

poor coördination, and certain types of ataxia, which may be due to involvement of the cerebellar efferent pathway.

3. Under suitable conditions these animals display extensor hypertonus of mild degree, which does not significantly interfere with locomotor or postural activities. It is suggested that the red nucleus alone cannot be held responsible for the inhibitory influences the removal of which causes decerebrate rigidity, but that this structure is merely a portion of the complex system regulating postural functions.

BIBLIOGRAPHY

- RADEMAKER, G. G. J. 1926. *Der Roten Kerne*. Berlin, J. Springer.
- INGRAM, W. R., S. W. RANSON, F. I. HANNETT, F. R. ZEISS AND E. H. TERWILLIGER. 1932. *Arch. Neurol. and Psychiat.*, xxviii, 513.
- INGRAM, W. R., S. W. RANSON AND F. I. HANNETT. 1932. *Journ. Neurol. and Psychopath.*, xii, 219.
- INGRAM, W. R. AND S. W. RANSON. 1932. *Arch. Neurol. and Psychiat.*, xxviii, 484.
- MUSSEN, A. T. 1927. *Brain*, l, 313.
- SPITZER, A. 1924. *Arb. a. d. Neurol. Inst. a. d. Wien Univ.*, xxv, 422.
- LORENTE DE NÓ, R. 1928. *Labyrinthreflexe*. Berlin, Urban & Schwartzberg.
- MAGNUS, R. 1924. *Körperstellung*. Berlin, J. Springer.
- HINSEY, J. C., S. W. RANSON AND R. F. McNATTIN. 1930. *Arch. Neurol. and Psychiat.*, xxiii, 1.

A TECHNIQUE FOR OBTAINING AND RECORDING ISOMETRIC CONTRACTIONS OF MAMMALIAN SKELETAL MUSCLES IN SITU

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In connection with a study of the energy metabolism of individual mammalian muscles in situ data on the isometric tensions developed by the muscles under investigation were required. The following technique was therefore developed.

The gracilis muscle of an anesthetized dog was freed from adjoining tissues with care not to injure its vascular and nervous connections. The tendon of insertion was dissected as far down the knee as convenient, transected, and secured to a specially designed clamp through which it was attached by a stout cord to a torsion wire myograph.

The clamp is illustrated in A, figure 1. The proximal end of the tendon was laid over the flat plate *a*, and the distal portion passed around the bar, *c*, and brought back beyond *a*, where it was secured by screwing plate *b*, firmly against *a*. The tendon was thus "snubbed" around *c*, obviating all danger of slippage when the clamp was properly tightened.

The myograph was constructed after the general design worked out in Sherrington's laboratory (Fulton, 1926). It is illustrated at B in figure 1. The myograph proper consists of a piece of piano wire approximately 1.5 mm. in diameter, *d*, secured immovably at one end to a metal block and resting snugly for 12 mm. of its length near its opposite end in a semi-cylindrical groove (which it fits exactly) in another metal block similar to the first. The length of wire between the blocks is exactly 50 mm. and the attachment to the muscle is exactly 5 mm. from the grooved block. Both blocks are fixed on a metal base which, in turn, slides in grooves in a metal frame, as indicated in B. The adjustment screw, *e*, allows the slack on the cord to be taken up and the initial tension of the muscle to be set as desired. The method of transmitting the torsion of the wire to a writing point is indicated in diagram C. The magnification used was about 50-fold. The resistance of the torsion wire was such that a tension of 4000 grams gave a record about 35 mm. in height. The myograph was carefully calibrated in grams per millimeter rise of the recording lever.

To avoid errors due to shifts in the position of the origin of the muscle

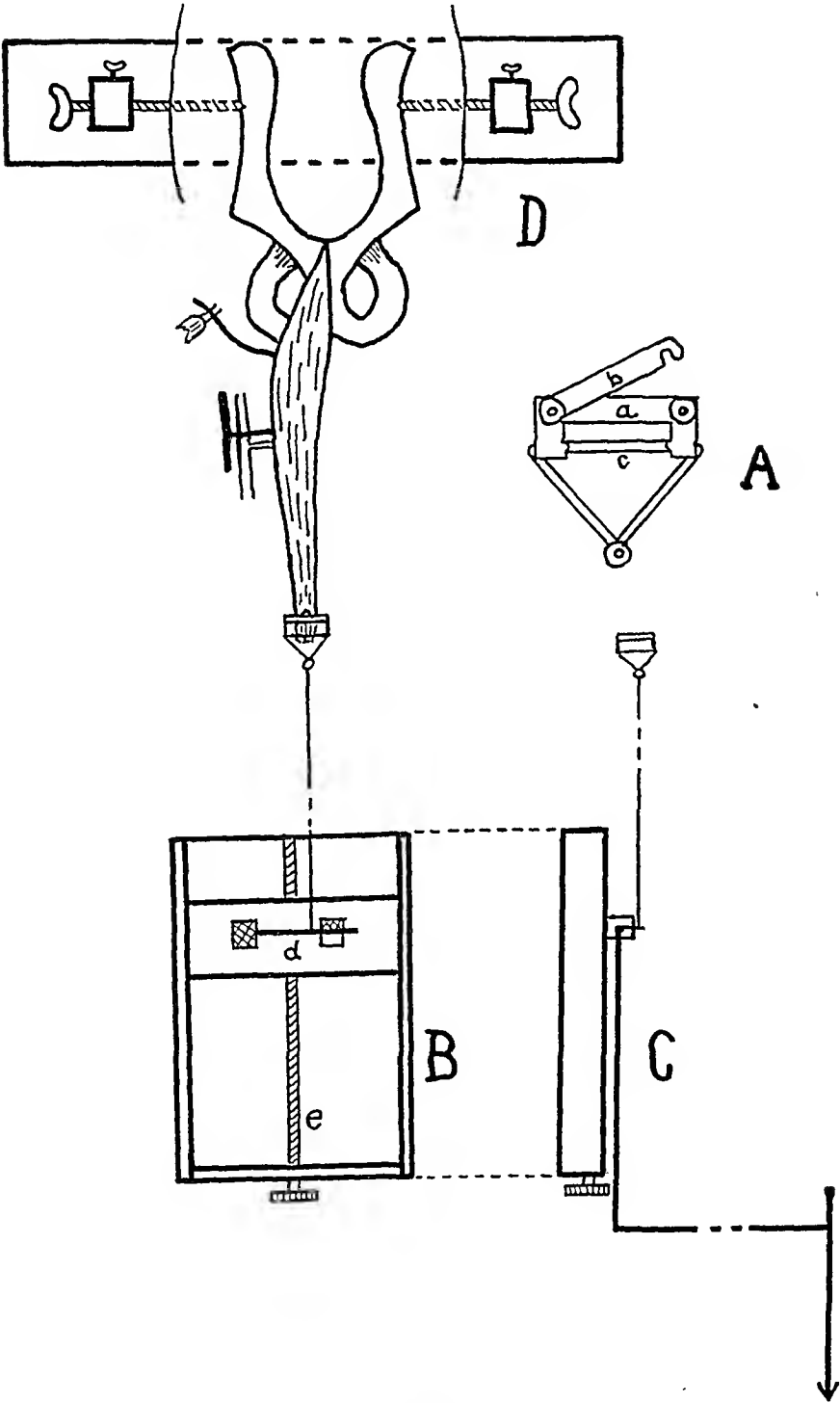


Fig. 1

the pelvis and leg were carefully immobilized. In the early experiments, modifying a technique of Liddell and Sherrington (1923), the pelvis was immobilized by passing a steel drill, 0.5 cm. in diameter and 45 cm. in length, transversely through both ilia, and securing the ends of the drill to the dog board. Satisfactory immobilization of the pelvis was thus secured, but we so frequently had the misfortune to damage an artery and lose considerable quantities of blood, besides finding it necessary to open the abdomen to tie off the ruptured vessel, that we devised a means of obtaining immobilization without penetrating the pelvis. The arrangement is shown at D, figure 1. It consists of a brass plate 45 cm. long by 10 cm. wide, on which are mounted two brass blocks, 20 cm. apart. Threaded bolts pass through the blocks as shown in D. These are 4.5 cm. above the base, and are pointed at their inner ends.

The procedure of immobilization was briefly as follows: With the dog in position on the board (a flat dog board was used in all this work) the outer surfaces of the two ilia were made accessible by incising the skin and overlying muscles. The brass plate was placed directly under the pelvis as indicated in the diagram. With the aid of guidance by the fingers the two bolts were screwed toward each other till their pointed tips engaged and held the two ilia. The bolts were then fixed in position by means of set screws, and the plate clamped to the dog board. With the pelvis thus immobilized the leg was drawn into line with the long axis of the body and tied firmly in place at knee and ankle.

Before attaching the insertion of the gracilis muscle to the myograph preparations were made for obtaining metabolic data (for details see accompanying paper); the gracilis branch of the obturator nerve was also exposed and a pair of shielded electrodes (vulcanite type) applied. The muscle was then attached to the myograph. The base carrying the torsion wire was screwed away from the muscle till all slack was out of the connecting cord and a slight initial tension (not exceeding 20 gm.) established.

Tetanic stimuli were applied to the nerve, the inductorium used being of the small Harvard type. Except in a few experiments the strength of stimulation was clearly supramaximal for the nerve trunk. The stimuli were interrupted rhythmically, periods of stimulation 0.5 second in duration alternating with periods of non-stimulation 0.7 second in duration, giving thus a stimulation rhythm of 50 per minute. This rhythm was selected as constituting a form of activity comparable to some types of maximum human exertion (in mounting stairs at top speed, four steps at a time, individual muscles contract about once a second and are probably in contraction about 0.35 second at each effort).

The primary purpose of the experiments was to obtain metabolic data (see accompanying paper) consequently the work periods were discontinued as soon as enough blood was collected to permit of determinations of oxy-

gen and sodium lactate concentrations in the blood. The work periods were therefore not of equal length, ranging between 0.5 minute and 3.0 minutes and averaging 1.3 minutes. Since the muscles contracted 50 times a minute the average number of contractions was 65. Satisfactory tension records were obtained in 39 experiments. In all of them the tension developed in response to stimulation fell off as the work period progressed, shading in the later stages more or less gradually into a "steady state." (In six experiments the contractions became too weak to move the writing lever to a measurable extent before a steady state was reached.) In twelve experiments the first dozen contractions (on the average) were of equal height or showed a definite "treppe," the later contractions falling off as described; in ten experiments the rate of decline was slow at first and subsequently became greater; in fifteen the rate of decline was strikingly uniform except for the shading into the steady state described above; in only two was the decline more rapid in the early stages of the period than later. The tension development during the steady state was at different levels in the different experiments, but the latter fell into two quite definite groups according to the amount of difference between the steady state and the initial activity. In the first group the tension development during the steady state averaged about 25 per cent of the initial maximum, while in the second group it averaged only 10 per cent of the maximum. The trend of activity was therefore either to a steady state in which individual contractions tended to be about one-fourth as powerful as the maximum of which the muscles were capable, or to a steady state in which the intensity of contraction averaged about one-tenth the maximum, or to a condition of complete exhaustion. The first trend appears to us more nearly typical of muscles functioning normally within the body for reasons which are presented in the accompanying paper.

Three alternatives suggest themselves as means of accounting for the decline in tension development. The first is the dropping out of motor units (Sherrington, 1925) through failure of excitation of some motor axons; the second is the dropping out of some of the muscle fibers within individual motor units through the onset of local "fatigue" of myoneural junctions; the third is fatigue of the muscle fibers themselves. As a matter of fact we have evidence that more than one of these factors may actually interact to bring about decline in developed tension. We have observed that a shift of the stimulating electrodes to a fresh area of nerve sometimes results in improvement in performance, proving at least part of the decline, in these cases, to be due to failure of excitation of some of the motor neurons, presumably through local "polarization" effects. We have not seen the initial tension fully regained by this procedure, consequently one or the other intramuscular factor must also operate to reduce the later contractions below the intensity of the earlier ones.

We plan to present in a subsequent communication some observations and interpretations bearing on the contractile powers shown by mammalian skeletal muscles in situ. The present paper is therefore confined to matter that is considered germane to the substance of the accompanying paper on the activity metabolism of muscles in situ.

SUMMARY

A technique is described whereby isometric contractions of dogs' gracilis muscles stimulated in situ through their nerves are recorded by means of a torsion wire myograph of special design.

Periods of activity composed of tetanic contractions of 0.5 second duration at a rate of 50 per minute, show a progressive decrease in tension development either from the first or after about a dozen contractions.

The trend of tension development is either toward a "steady state" in which the muscle continues to develop an approximately constant tension during a long series of contractions or toward a condition of "exhaustion" in which not enough tension is developed to be measured on the tracing.

BIBLIOGRAPHY

- FULTON, J. F. 1926. Muscular contraction and the reflex control of movement. Baltimore.
- LIDDELL, E. S. T. AND C. S. SHERRINGTON. 1923. Proc. Roy. Soc. B, xcv, 143.
- SHERRINGTON, C. S. 1925. Proc. Roy. Soc. B, xcvi, 519.

THE ACTIVITY METABOLISM OF MAMMALIAN SKELETAL MUSCLE IN SITU¹

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The studies of muscle metabolism which have been in progress in this laboratory since 1926 have been concerned primarily with the processes in which production of lactic acid is a predominant feature. The interpretation placed upon these processes has recently undergone profound modification, consequent on the discovery of creatine phosphoric acid (Fiske and Subbarow, 1927; Eggleton and Eggleton, 1927) and of the occurrence of normal contractions in muscles poisoned with monoiodoacetic acid without any concurrent production of lactic acid (Lundsgaard, 1930), but for the basic problem of relating the chemical energy turnover occurring in muscle in situ to the potential or actual yield of mechanical work, studies of oxygen consumption and lactic acid production continue to constitute the most logical and practical method of attack. The precise thermodynamic determinations of A. V. Hill have been carried out mainly on excised muscles of the frog. Meyerhof's chemical studies have been made chiefly on similar material. We have performed our experiments on anesthetized dogs with circulation through the muscles studied as nearly unimpaired as possible.

METHODS. As in our work previously reported (Martin, Field and Hall, 1929) amytal anesthesia was used. Continuous records of arterial blood pressure were obtained from one carotid, using an ordinary mercury manometer. Continuous records of oxygen consumption were also made with the aid of a Sanborn "grafic" metabolism apparatus. Readings of body temperature were taken at sufficiently frequent intervals to enable us to plot a continuous approximate record of body temperature.

The experiments to be reported in this paper fall into three groups, according to the treatment to which the muscles were subjected. The first group (HBI) includes experiments in which no activity was induced. In

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the second group (KL) a single muscle (gracilis) was worked in situ by rhythmic excitation of the gracilis branch of the obturator nerve. In the experiments of the third group (J) the main muscles of one leg were worked similarly, stimulating electrodes being placed on the femoral, sciatic and obturator nerves, each of which was sectioned central to its electrodes.

In all the experiments the muscles whose lactate content was to be determined were prepared by separating them from adjoining tissues as fully as possible without disturbing any vascular or nervous connections and without cutting any attachments. This work was done early in the experiments; the muscles were then covered with gauze soaked in warm Ringer's fluid and the skin drawn back into place. At the desired moment the test muscles were frozen in situ with carbon dioxide snow according to a method suggested by Davenport and Davenport (1928) and were then excised and thrown into liquid air. (In some of the experiments the CO₂ snow freezing was omitted from one or more muscles for purposes of comparison.) The muscle substance, frozen in liquid air, was ground finely in a mortar; five grams were then weighed out and suspended in water. Proteins were precipitated by the copper sulphate tungstate method (M. Somogyi, 1931) and carbohydrates by the method of Van Slyke (1917). The subsequent analytical treatment was the same as used by us for the determination of blood lactates (Martin, Field and Hall, 1929). Arterial blood samples were taken through a cannula placed in the carotid not in use for the arterial pressure record. These were analyzed for lactates by the procedure indicated above.

Blood and muscle lactates in resting dogs. To assure ourselves that observed changes in lactate content following activity were properly attributable to the activity and not merely consequent on the passage of time the experiments of our first group were done. The only operative procedures additional to those previously described consisted in exposing one brachial and one femoral vein, and in some of the experiments still other large veins, to permit the withdrawal of samples of venous blood, usually by simple venopuncture with syringe needles.

About ten minutes were allowed to elapse after completing the operative preparations to permit the records of arterial blood pressure and oxygen consumption to get well under way. Samples of blood (about 5 cc. in volume) were then drawn from an artery, a brachial vein and a femoral vein, and one or sometimes two flexor muscles of the foreleg were frozen in situ and excised. These tasks were completed within about fifteen minutes ordinarily. A further wait was then allowed of fifteen minutes to a half-hour, followed by freezing and excision of one hind leg muscle (gracilis or rectus femoris; in a few instances a gastrocnemius). In about half the experiments a fore leg muscle was also excised at this time. A further period of at least a half-hour was then allowed to pass. The third and final

set of samples was then taken, including hind leg muscles in nearly all the experiments, fore leg muscles in a few, and arterial and venous blood samples in nearly all.

Our findings in respect to lactate content are summarized in table 1. They show quite clearly that no great changes in either blood or muscle lactates occur in amyotomized dogs during the period, usually not exceeding an hour and a half, occupied by our activity experiments of the subsequent groups.

Some incidental findings are of enough interest to justify brief comment. It will be noted that the lactate contents of brachial vein blood and femoral

TABLE 1

Muscle and blood lactates in resting dogs

All figures in milligrams per cent lactic acid.

	MEAN	RANGE
Early samples		
Arterial blood.....	24.4*	6.8-52.5
Femoral vein blood.....	21.0	10.8-33.0
Brachial vein blood.....	23.6	11.6-59.0
Flexor muscle of fore leg.....	48.4*	15.6-90.6
Samples one-half to one hour later		
Fore leg muscles.....	35.0	19.1-46.4
Hind leg muscles.....	40.7	23.7-63.2
Samples more than one hour after the first		
Arterial blood.....	29.6	13.3-46.8
Venous blood.....	35.4	16.9-76.0
Fore leg muscles.....	43.5	28.6-59.2
Hind leg muscles.....	56.5	14.0-86.0

* Data from the entire series of experiments are included.

vein blood averaged about the same. This is neither a novel nor an unexpected observation, but is worth recording as supporting the idea that the basal metabolic processes in muscles in different regions tend to be pitched on about the same scale. In general high arterial blood lactates are associated with high venous blood lactates and both with high muscle lactates. The correspondence is less close, however, than would perhaps be anticipated, the Pearson coefficient of correlation between arterial and venous blood lactates being only 0.75 ± 0.046 ; between venous blood lactates and muscle lactates 0.70 ± 0.055 , and between arterial blood lactates and muscle lactates 0.060 ± 0.064 . In assembling the above data none

were paired unless they were obtained from samples taken within a few minutes of each other. For the correlation between arterial and venous lactates the mean of the values obtained from brachial and femoral vein bloods was taken as representing the approximate composition of mixed venous blood. For correlating muscle lactates with venous blood lactates fore leg muscles were paired with brachial vein blood and hind leg muscles with femoral vein blood.

The occasional lack of correspondence between arterial and venous blood lactates noted above has been pointed out by other workers (Himwich, Koskoff and Nahum, 1930) and we have considered one aspect of it in another place (Martin, Field and Hall, 1930a). We were led by it to perform a few experiments in an attempt to locate the points in the circulation at which bloods with quite different lactate contents become intermingled. Our argument was that if the blood in the femoral vein, for example, has a high lactate content, while arterial blood has a low lactate content, not only must the hind leg muscles be contributing lactates to the blood, but at some point in the course of the blood from the femoral vein toward the left ventricle it must become diluted with blood of a decidedly lower lactate content. (The possibility that the heart muscle may withdraw lactates directly is also not to be overlooked.) A practical difficulty confronting us in this work is that we have no means of knowing during individual experiments whether the arterial and venous lactate values are near together or far apart, the lactate analyses not being completed until some hours after the acute experiments are over.

In these experiments we drew venous blood through a well vaselined male urethral catheter, which was passed into the venae cavae by way of the right external jugular vein. Careful measurements were made on the basis of which we estimated how far the catheter must be inserted to bring its tip successively into the superior vena cava, the inferior vena cava above the entry of the hepatic vein, the same between the hepatic and renal veins, and finally the same below the entry of the renal veins and above the bifurcation. All locations were checked *post mortem* in each experiment and found to have been successfully obtained. Our most striking experiment was one in which the lactate content of arterial blood was 15.5 mgm. per cent, while blood from the inferior vena cava below the renals showed a lactate content of 42.2 mgm. per cent. The sample from the same vein above the renals and below the hepatic had 35.1 mgm. per cent lactate; the sample from above the hepatic, 19.3 mgm. per cent, and the sample from the superior vena cava 20.9 mgm. per cent. In this experiment the liver was certainly contributing blood of low lactate content. Whether it was actually withdrawing lactates from the blood as Himwich and co-workers have shown it to do in many cases (*loc cit.*, 1930) cannot be stated with certainty from our data, although the presumption is that

it was doing so. In another experiment the lactate content of arterial blood was 15.8 mgm. per cent and of inferior vena cava blood from below the renals 23.0 mgm. per cent. A sample of blood taken directly from a renal vein contained 29 mgm. per cent lactic acid, and blood from the inferior vena cava above the hepatic 27.5 mgm. per cent. However, blood from one brachial vein contained 15.3 mgm. per cent lactate, and from a subclavian vein only 9.6 mgm. per cent. In this case the forward end of the body was contributing the blood of low lactate content by which the arterial blood took on a lower lactate value than postcaval blood. In four other experiments there proved to be no significant differences in lactate content between arterial blood and blood from the inferior vena cava at the different levels from the bifurcation forward.

TABLE 2

Data on oxygen consumption, rectal temperature and arterial pressure from the experiments of the first group (19 experiments)—resting dogs

PERIOD	OXYGEN CONSUMPTION PER KILOGRAM PER MINUTE		RECTAL TEMPERATURE		ARTERIAL PRESSURE	
	Mean	Range	Mean	Range	Mean	Range
1*	5.4	2.6-7.5	37.6	34.0-39.8	99	56-158
2	5.3	3.8-7.0	37.7	34.6-41.2	93	70-125
3	5.3	3.9-7.2	37.6	34.6-41.6	86	62-123
4	5.3	3.8-6.5	38.2	35.0-41.6	82	64-118

The periods represent half-hour intervals.

The first period began immediately upon completion of the operative preparations. It varied from one hour to about two and one-half hours after administration of amytal.

* The data of the first period include all the experiments reported in this paper. The mean values for the experiments of the first group taken by themselves agree closely with those in the table although the ranges differ slightly.

Our observations on oxygen consumption, rectal temperatures and arterial blood pressures in the experiments of this group are summarized in table 2. No special comment is necessary except in connection with the arterial pressure data, which show a steady decline in arterial pressure with the passage of time. The experiments described in this paper did not exceed two hours in length after the starting of the records of arterial pressure, and within this period the decline does not assume significant proportions; but it is clear that in prolonged experiments in which amytal is used as anesthetic possible effects of the drug on arterial pressure should be taken into account.

Metabolic data from single muscles. In the experiments of the second group (KL) the gracilis muscle of each side was separated from its adjoining tissues, the gracilis branch of the obturator nerve was exposed, and also

the femoral artery and vein for a short space both above and below the origin of the main nutrient vessels of the muscle. Great care was taken to avoid injury to or compression of these latter. All minor veins in the exposed area, other than the one communicating with the gracilis muscle, were tied off. A cannula directed centrally was inserted into the femoral vein just below the point of union of the gracilis vein. The cannula and femoral vein up to the gracilis vein were then filled with a thin colorless oil (petrolatum liquidum).

After the above preparations were complete the pelvis and one leg were carefully immobilized and the tendon of the gracilis muscle on the immobile side was cut and attached to a torsion wire myograph (for details of the latter and of the technique of immobilization see accompanying paper).

After these preparations were finished a well vaselined syringe was attached to the cannula in the femoral vein and at a given signal the same vein was clamped off central to the point of entry of the gracilis vein. All the blood entering the femoral vein from the gracilis muscle was thus diverted into the syringe, and the total resting blood-flow for any desired short period collected. No special effort to avoid clotting was used other than the well greased cannula and syringe. The withdrawal of blood was continued until enough was obtained for determinations of oxygen content (by the method of Van Slyke) and of blood lactate content. The volume and time were carefully noted. The volumes averaged 10 ml. and the periods 3 minutes. The muscle was then worked by administering 50 brief tetanic stimulations per minute to the gracilis branch of the obturator nerve (for details see accompanying paper). The resultant isometric contractions were recorded on a slowly moving drum. During the work period the venous blood from the muscle was collected in the same manner as described above. Our practice was to continue the stimulation until as much blood was obtained as needed for the oxygen and lactate determinations. In some cases thirty to forty-five seconds sufficed, in two or three nearly three minutes were required; the majority were worked 1 to 1.5 minutes.

At some convenient stage in the proceedings, usually just before withdrawing the first sample of resting venous blood, a sample of arterial blood was taken, and the rectus femoris muscle on the same side was frozen in situ with carbon dioxide snow, excised and ground to powder in liquid air to be analyzed as a control on the lactate content of the resting gracilis muscle. Immediately upon completion of the stimulation the worked gracilis (whose length had been measured in the meanwhile) was similarly treated and excised. The portions of muscle not used for analysis were collected and the weight of the whole recorded. All these procedures were then repeated on the opposite side of the body.

At the end of each experiment the following data were in hand: the lac-

tate content and oxygen content, first of arterial blood, second, of the venous blood circulating in a known time through a resting gracilis muscle of known length and weight, and third, of all the venous blood that passed through the same muscle during a known brief period of work; also the volume of blood-flow during a known resting period and the entire work period; also the lactate content of the gracilis muscle at the end of the work period; and to give an idea of the lactate content of the gracilis prior to stimulation, the corresponding datum from an adjoining (rectus femoris) muscle in the resting state. The tension developed by the worked muscle at each contraction and the total number of contractions were also known. The quantities of oxygen present in blood were expressed in cubic centimeters per 100 ml.; of lactate in blood in milligrams lactic acid per 100 ml.; and of lactate in muscle in milligrams lactic acid per 100 grams of muscle tissue.

When attempts were made to summarize the metabolic data in tabular form we encountered the fact (considered in detail in the accompanying paper) that in every experiment the tensions developed in successive contractions declined more or less rapidly from a maximum registered at or near the beginning. It is obvious that the metabolism of the active muscles must also have declined as the work continued, and more or less parallel with the decline in developed tension (see p. 490). In view of this decline, and also of the variation in length of the work periods in the different experiments the findings cannot be satisfactorily summarized in terms of the usual arbitrarily selected time unit, of one minute, but must be presented rather in terms of the time that actually elapsed in the work periods. This time ranged from 0.5 minute to 3.0 minutes, averaging 1.3 minutes. Table 3 accordingly presents our metabolic data averaged in terms of this mean work period. The gracilis muscles studied in these experiments ranged in weight from 12.5 grams to 80.0 grams, averaging 44.0 grams. In length the range was from 9 cm. to 21 cm., with an average of 16 cm. The maximum tension developed by individual muscles ranged from 1810 grams to 6200 grams, averaging 3400 grams.

Metabolic data from experiments in which one hind leg was worked. In our third group of experiments the main muscles of one hind leg were stimulated as described on page 482. Control samples were taken from the opposite hind leg. The series is short, consisting of but nine experiments. The data on blood and muscle lactates are summarized in table 4. Neither the rectal temperature nor the arterial blood pressure were significantly altered by the amount of work given these dogs. The mean pre-work temperature was 37.3°C.: the mean at the end of activity 37.5°C.; and the mean for the succeeding half-hour period 37.2. The mean arterial pressures were: pre-work, 105 mm. Hg; during work, 100 mm. Hg; during the succeeding half-hour period, 92 mm. Hg.

The oxygen consumption of the whole animal (table 5) increased de-

cisively in every experiment within a few seconds of the beginning of stimulation, and the consumption rate rose rapidly to its maximum (within the limit of error of the determinations). In two of the experiments (4 and 7R) the increase in oxygen consumption was disproportionately large, and

TABLE 3
Metabolic data from single gracilis muscles worked in situ (50 experiments)
Mean work period = 1.3 minutes

	MUSCLE AT REST		MUSCLE WORKED	
	Mean	Range	Mean	Range
Blood flow per 100 grams muscle per minute.....	9.0	4.0- 21.8	21.5	11.8- 46.0
Blood flow per 100 grams in 1.3 minutes.....	11.8		28.0	
Oxygen content of arterial blood (cc. per 100 ml.).....	20.0	12.8- 25.4	20.0	
Oxygen content of gracilis vein blood (cc. per 100 ml.).....	9.5	3.1- 16.2	6.8	1.6- 12.8
Mean arterio-venous oxygen difference.....	10.5		13.2	
Mean oxygen consumption per 100 grams muscle in 1.3 minutes.....	1.2 cc.		3.7 cc.	
Excess oxygen consumption of 100 grams muscle due to 1.3 minutes' work.....			2.5 cc.	
Lactic acid content of arterial blood (mgm. per cent).....	29.0	14.8- 56.7	29.0	
Lactic acid content of gracilis vein blood (mgm. per cent).....	30.0	15.4- 48.9	37.6	20.4- 69.1
Amount of lactic acid entering 28.0 ml. blood (mean blood-flow of worked muscle).....			2.4 mgm.	
Lactic acid content of gracilis muscle (mgm. per 100 grams).....	60.0*	34.1-133.0	160.0	64.4-284.0
Increase in lactic acid per 100 grams muscle during work.....			100.0 mgm.	
Total production of lactic acid in 100 grams muscle due to 1.3 minutes' work.....			102.4 mgm.	

* From rectus femoris muscles taken as controls.

while we do not doubt that the increase was as genuine, and as much due to the stimulation in these as in the other experiments, it seems that a more nearly typical picture can be constructed if we omit them from our averages, which we have accordingly done. The means obtained from our data indicate, therefore, that vigorous rhythmic tetanization of the muscles of

one hind leg in dogs with an average weight of 18.4 kgm. will tend to increase the oxygen consumption from an average of 99 cc. per minute (5.4 cc. per kgm. per min.) to an average for the first three-minute period of 156

TABLE 4

Muscle and blood lactates in experiments in which the muscles of one hind leg were given rhythmic indirect stimulation for 15 minutes

All figures in milligrams per cent lactic acid.

	ARTERIAL BLOOD LACTATES		FEMORAL VEIN BLOOD LACTATES (WORKED SIDE)		FEMORAL VEIN BLOOD LACTATES (NON-WORKED SIDE)	
	Mean	Range	Mean	Range	Mean	Range
Before work	27.1	19.7-33.8	29.8	19.1-43.5	32.2	20.4-43.4
At the end of work	45.0	35.4-55.4	56.5	45.8-74.0	40.7	38.0-43.3
1 hour after work	38.0	30.0-49.2	42.0	31.2-51.5		

Muscle lactates

	WORKED MUSCLE		NON-WORKED MUSCLE	
	Mean	Range	Mean	Range
Before work			68.5	58.2-78.0
At end of work	148.0	96.0-253.0	80.0	69.0-91.0
1 hour after work	84.0	77.8- 89.0		

TABLE 5

Oxygen consumption data from dogs in which all main muscles of one hind leg were worked

	MEAN	RANGE
Weight of dogs	18.4 kgm.	14.5-21.0 kgm.
Pre-work oxygen consumption	99	80 -130 (cc. per minute)
Oxygen consumption during work:		
1st three minutes	156	130 -201 (cc. per minute)
2nd three minutes	134	109 -171 (cc. per minute)
3rd three minutes	131	108 -166 (cc. per minute)
4th three minutes	127	98 -157 (cc. per minute)
5th three minutes	121	88 -147 (cc. per minute)
Oxygen consumption following work:		
1st half-hour	100	77 -120 (cc. per minute)

cc. per minute. The maximum oxygen consumption is reached practically at the beginning of the activity, and too soon, so far as we can judge, for any secondary factors to be brought into action through the circulating blood in parts of the body outside the active leg. We therefore attribute

the whole of the early increase in oxygen consumption to the muscles actually thrown into contraction by the stimulation. The weight of these muscles was determined directly in one dog (J4) immediately after the death of the animal, and found to be approximately 1500 grams. This dog weighed 26.3 kgm. In a dog weighing only 18.4 kgm., which is the mean on which our calculations are based, the corresponding muscles can hardly weigh more than 1200 grams. It would appear, therefore, that the immediate increase in oxygen consumption amounting on the average to 57 cc. per minute must be due wholly to the activity of not more than 1200 grams of muscle tissue, corresponding to about 4.7 cc. per 100 grams of muscle per minute. It will be noted that this is a considerably larger average oxygen consumption than we found in our experiments with single muscles. The interpretation of this difference is considered in the discussion.

DISCUSSION. *The partition coefficient for lactates.* The experiments of the first group (muscles not worked) call for no particular discussion beyond mention of the fact brought out in table 1, and reappearing in tables 3 and 4, that the lactic acid content of resting muscles tends to be about double that of the venous blood emerging from them. The same ratio also appears in experiments on worked muscles in which enough time has elapsed to permit equilibrium to be reestablished (table 4). A coefficient of 2 can therefore be considered to express the partition ratio for lactates between the muscles and blood of dogs.

The metabolic basis of brief tetanic contractions. In the experiments of the second group in which single muscles were worked in situ the primary problem is to relate the oxygen consumption and lactic acid production to the initial energy-yielding process, now generally conceived to be the explosive decomposition of phosphocreatine (creatine phosphoric acid). The muscles were subjected to successive brief tetanizations of equal duration, the stimulation rate of each tetanus being rapid enough to ensure complete fusion of the component contractions. The work of Hartree and Hill (1921) and of Feng (1931) permits the generalization that in unfatigued muscles stimulated thus the tensions developed in the successive contractions bear a constant ratio to the initial heats, which, in turn, are conceived to be directly proportional to the quantity of phosphocreatine decomposed (Hill, 1932). The relative tensions developed in the successive isometric contractions of a given work period may therefore be considered indicative of the relative amounts of phosphocreatine decomposed during the same contractions, at least while the muscle remains unfatigued. In the later stages of work periods, after marked fatigue has developed, the relationship is in question, Feng (loc. cit.) having shown that when fatigue is present, slowing the response of the muscle, the ratio of tension to initial heat rises. According to Bronk (1930), however, the rise in the ratio is seen only in

experiments in which the frequency of stimulation is such that fusion is incomplete prior to the onset of fatigue and becomes complete as fatigue develops. Feng appears to consider his results comparable to and confirmatory of Bronk's, consequently the greater economy of contraction of fatigued as compared with fresh muscles cannot be considered to be established for all stimulation frequencies, and may conceivably not apply to the conditions of our experiments, in which complete fusion was present at all stages.

Disregarding for the present this possible consequence of fatigue we may therefore analyse our experiments on the principle that the relative tensions developed in the successive isometric contractions of a given work period are reliable indices of the relative amounts of phosphocreatine decomposed in the same contractions.

The resynthesis of phosphocreatine. Virtually coincident with the decomposition of phosphocreatine is the beginning of its resynthesis. In its first stages this resynthesis is largely or wholly oxidative, provided oxygen is available (Eggleton and Eggleton, 1928). In the absence of oxygen, or if the supply of the same is insufficient, a supplementary process involving lactic acid production comes into play (see Hill, 1932, for summary and citations). That portion of the resynthesis which depends on lactic acid production appears to be slower than the oxidative portion in that, in frog's muscles, production of delayed anaerobic heat sometimes continues for as much as three minutes after contraction is over (Hartree and Hill, 1920). The total energy of the restorative reactions is slightly in excess of the energy cost of resynthesizing the decomposed phosphocreatine, as shown by the invariable liberation of a small quantity of delayed anaerobic heat as just indicated, but is nearly enough equivalent to justify the conclusion that the restorative reactions are governed either directly or indirectly by the decomposition of phosphocreatine.

The energy-magnitude of the restorative reactions attending any isometric contraction may therefore be looked upon as in direct ratio to the quantity of phosphocreatine decomposed, which in turn is in direct ratio to the tension developed. The last thus serves as an index to the first, entitling one to interpret data on oxygen consumption and lactic acid production in terms of the relative tensions developed during the successive contractions of a work period.

Delayed lactic acid production in mammalian muscles. The ratio considered just above is valid only for the sum total of the restorative reactions, hence is applicable to cases in which lactic acid production is involved only when the delayed production is included along with the immediate production. It becomes necessary, therefore, to account for the delayed production in order to use tension development as an index of metabolism. We offer the following considerations as showing that the delayed acid pro-

duction is accounted for in our work. The argument depends for its validity on three assumptions, all of which have evidence, either direct or indirect, in their support. These assumptions are: first, that in mammalian muscles at body temperatures the delayed acid production is not so long drawn out as in frog's muscles, but ends within not much more than one minute at the most instead of requiring three or thereabouts; second, that the act of tension development has no effect *per se* on the production of lactic acid, so that the incidence of subsequent contractions does not affect the delayed production of acid consequent on earlier contractions except as such production is influenced in general by the quantities of decomposed phosphocreatine and of lactic acid present in the system; third, that oxidative resynthesis of phosphocreatine takes precedence over resynthesis due to acid production, and to such an extent that in the presence of adequate oxygen little or no production of lactic acid takes place.

If these assumptions are sound the determinations of lactic acid given in tables 3 and 4 include the delayed as well as the immediate production, and therefore give valid data on which to base interpretations of tension records. This conclusion follows from the fact that in an average work period, such as must be taken as the basis on which to interpret mean metabolic data, the developed tension falls off markedly during the later stages (see accompanying paper). The decomposition of phosphocreatine declines in parallel ratio, reducing correspondingly the accretion of additional amounts requiring resynthesis. The oxygen supply becomes more and more nearly adequate to care for these accretions as they become progressively less (see below), thus the need for additional immediate production of acid falls well toward or quite to zero. The power of the muscle to produce lactic acid is not, however, exhausted (in no case have we seen in muscles worked by successive brief tetanizations as high concentrations of acid as were observed by Eggleton and Evans (1930) after continuous tetanization for several seconds). Hence the delayed production referable to the early part of the work period, when the requirement for acid is at its peak, is to be presumed to be largely met during the later part of the period; meanwhile the delayed production referable to the later part has become so small that the time required for chilling the muscle with carbon dioxide snow (amounting to at least a minute) is adequate to permit all or virtually all of the residual delayed acid production to take place. We conclude, therefore, that our worked muscles, when analyzed, contain the whole amount of lactic acid called for by the quantity of phosphocreatine decomposed.

The theoretical oxygen requirements in brief tetanic contractions. In the experiments under consideration an average work period included 65 tetanic contractions, which were of unequal value (at least after the first few), becoming progressively less powerful as the work proceeded until either

exhaustion or a "steady state" was reached. If the contractions had all been of equal intensity (and assuming no change in "efficiency" to have occurred) one sixty-fifth of the total oxygen consumption and an equal proportion of the total lactic acid production would properly be attributable to each contraction. Table 3 shows that in an average work period 100 grams of muscle used 2.5 cc. of oxygen (above the resting requirement) and produced 102.4 mgm. of lactic acid. The proportional share for each contraction would therefore be 0.038 cc. of oxygen and about 1.55 mgm. of lactic acid. According to the calculations of A.V. Hill (1926, p. 88) the removal of 1 mgm. of lactic acid by oxidation implies the consumption of approximately 0.143 cc. of oxygen. If sufficient oxygen could be consumed during work to support the entire energy metabolism, the amount corresponding to the above lactic acid production would be about 0.222 cc. This, added to the amount consumed directly, gives a total of 0.26 cc. as the "net theoretical oxygen requirement," or net metabolic cost, of a single average contraction of 100 grams of muscle in the experiments of this series. The oxygen requirement of the resting muscle for the period of a single contraction cycle is about 0.02 cc. Hence the "total theoretical oxygen requirement" for a single average contraction is about 0.28 cc. per 100 grams of muscle. At the contraction rate used in our experiments this corresponds to a theoretical requirement of 14 cc. of oxygen per 100 grams per minute.

Our first step in comparing the metabolic costs of individual contractions with the metabolic cost of the average contraction of the series was to determine to what extent the greatest contraction of a given work period exceeded the mean contraction of the same period. In 21 experiments the ratio of greatest tension to mean tension ranged between 1.20 and 2.16, averaging 1.5. Since the average "net theoretical oxygen requirement" was 0.26 cc., this implies a "total theoretical oxygen requirement" for the contractions of greatest intensity of the order of 0.41 cc. This theoretical requirement was met in part by a consumption of oxygen during the contraction (and the immediately following brief period of relaxation) and in remaining part by excess production of lactic acid, some of which occurred during the period of the contraction and some of which was delayed. A method of deducing the immediate oxygen consumption is afforded by an analysis of the blood supply. We accordingly turn to such an analysis at this point.

The blood supply of individual fibers. A study of the capillary circulation in the gracilis muscles of dogs has recently been made in this laboratory (Martin, Woolley and Miller, 1932). The conditions of rest and activity of the test muscles were purposely made to correspond as closely as possible with the corresponding conditions in the muscles studied in the present series, hence the findings in that study are fully applicable to this. The

study shows that in the resting muscle at any given instant about sixty per cent of the fasciculi have open capillaries, and that within each fasciculus with open capillaries the ratio of number of muscle fibers to number of open capillaries is about three to two. It follows that in the muscle as a whole the effective ratio of fibers to open capillaries is about two to one. The same study shows that when the muscle is worked open capillaries are present in nearly all of the fasciculi and the number of open capillaries per fasciculus is about doubled. Consequently the ratio of fibers to open capillaries becomes about three to four, the muscle as a whole showing about 3.3 times as many open capillaries as during rest. Furthermore, studies by Miriam Miller (unpublished), of the size of individual capillaries in worked as compared with resting muscles, have indicated the average increase in diameter to be of the order of 15 per cent, corresponding to an average increase in cross section of about 32 per cent. The net effect of the interaction of the above factors is to increase the width of the capillary bed by about $4\frac{1}{2}$ -fold, and this we consider the probable maximum increase under the conditions of our experiments. As pointed out in the paper cited above, a further increase (presumably of humoral origin and amounting conceivably to an approximate doubling) may be possible through the introduction of positive capillo-dilatation, but this implies other conditions than were present in the experiments here under discussion.

The blood supply to a muscle can also be increased by an increase in velocity of the blood flow through individual capillaries. In the present series of experiments, however, no increase in velocity of capillary flow occurred. On the contrary the increased blood flow through the muscle as a whole was only about $2\frac{1}{2}$ times the resting value (see table 3), considerably less than the potential increase due to widening of the capillary bed. In connection with this observation we would call attention to the fact that there is a certain latency in the attainment of the maximum width of capillary bed (Martin, Woolley and Miller, loc. cit.). The latency is not sufficiently pronounced to prevent a striking increase in rate of blood flow from being manifest at the very beginning of activity, but presumably does operate to delay somewhat the attainment of maximum flow. It follows that the maximum increase in rate in our experiments may be supposed to have been somewhat greater than the total increase, given above as $2\frac{1}{2}$ times the resting rate. No data are available as to the amount of difference, but observations presented below make it appear unlikely that the difference was sufficient to make the maximum rate exceed the resting rate by as much as the $4\frac{1}{2}$ -fold suggested above as the maximum probable increase in width of capillary bed.

The delivery of oxygen to active fibers. Another factor providing an increase in the supply of oxygen to active fibers is a greater dissociation of oxyhemoglobin. In our experiments the average discharge of oxygen into

the worked muscles was 13.2 cc. per 100 ml. of blood, as compared with a mean discharge of 10.5 cc. per 100 ml. into the same muscles before excitation (table 3), an increase of approximately one-third. Combining this with the maximum increase in width of the capillary bed estimated above we have a potential maximum increase in delivery of oxygen to individual fibers during activity amounting to nearly six-fold.

Other investigators (Verzar, 1912; Himwich and associates, 1927 and 1929) have reported a utilization of oxygen per 100 ml. of blood by active muscles of the same order as we have observed, but a considerably less utilization by muscles at rest. Consequently their data indicate a larger proportionate increase in oxygen delivery due to increased oxyhemoglobin dissociation; an approximate doubling, in fact. If their data on oxyhemoglobin dissociation are combined with ours on widening of the capillary bed a potential increase in oxygen delivery of about nine-fold is indicated. This figure seems to us significant in that it suggests the limit that will presumably be encountered in experimental studies of muscular metabolism of the type here under discussion. (The above limit was approached in the results reported by Himwich and associates, 1927, 1929; much less nearly in the work of Verzar, 1912, of Barcroft and Kato, 1916, and in the experiments of the present series.)

Oxygen deficiency. At the moment stimulation is begun the muscle is in the resting state, receiving the blood-flow characteristic of that state and consuming oxygen at the resting rate, which in our experiments averaged 0.02 cc. O_2 per 100 grams muscle for the time occupied by a single contraction. The first contraction is always so nearly the maximum for the series that for the purposes of the present discussion it may be considered wholly so (see accompanying paper). An average total theoretical oxygen requirement of some 0.41 cc. is therefore established in each 100 grams of muscle with no immediate provision for meeting it beyond the limited provision afforded by an increase in the dissociation of oxyhemoglobin. In our experiments this factor increased the oxygen supply by only about one-third, hence before widening of the capillary bed begins to become effective the total available oxygen will not exceed 0.03 cc. per 100 grams of muscle to meet a theoretical requirement of about 0.41 cc. Coincident with the widening of the capillary bed the amount of available oxygen increases, reaching a maximum probably fairly early in the work period. Since in our experiments the average oxygen consumption for the entire work period was approximately three times the resting consumption (table 3) and the rate of consumption (parallel in general with the rate of blood flow) rose rapidly during the early stages of the period, the value of the maximum could not have been greatly in excess of the average. We postulate it, for purposes of illustration, at four times the resting figure. During the early part of an average series of 65 contractions the average oxygen utilization

per 100 grams of muscle per contraction is conceived, therefore, to have risen from about 0.03 cc. to about 0.08 cc. The theoretical oxygen requirement, on the other hand, is estimated to have declined by the end of the period from 0.41 cc. per contraction to a final value of the order of 0.10 cc. per contraction, the latter figure being computed from the average tension developed during a typical "steady state" (see accompanying paper).

The theoretical oxygen requirement *not met by oxygen* declines, according to the above calculations, from about 0.38 cc. per 100 grams of muscle per contraction at the beginning of exercise to about 0.02 cc. at the onset of the steady state. This part of the requirement is actually met by excess production of lactic acid. The equivalent quantities of acid for the above maximum and minimum are about 2.7 mgm. and 0.14 mgm. respectively.

A significant conclusion implied in the calculations given above is that whenever a skeletal muscle as a whole or *any group of fibers within it* contracts tetanically in the body a theoretical oxygen requirement is incurred which considerably exceeds the potential delivery of oxygen to the contracting fibers by the blood. This conclusion appears unavoidable in view of the maximal character of all contractions of individual fibers (under usual conditions of stimulation) considered in connection with the arrangement of the capillaries within the muscle, which is such as to localize sharply the potential sources of oxygen for individual fibers.

A second conclusion is that the greatest oxygen deficiency is necessarily incurred at or near the beginning of activity. This conclusion follows in part from the lag in widening of the capillary bed which has the effect of affording the muscle fibers a better oxygen supply after the exercise is well under way than at its very beginning, and in part from the reduction in force of contraction, and hence in quantity of phosphocreatine to decompose as activity continues. The above conclusions imply that when the contractions follow one another in quick succession, as in our experiments, the oxygen deficit mounts rapidly at first. We also have evidence that even though the production of lactic acid may not be rapid enough to meet the deficit as incurred the lactate concentration of the muscle also mounts rapidly. We have reported elsewhere experiments in which high concentrations of muscle lactate were found to be present after 30 seconds of rhythmic excitation (Martin, Field and Hall, 1930b), and we have seen in still briefer work periods, of 10 and 15 seconds respectively, increases in lactic acid concentration of 28 and 42 mgm. per cent, corresponding to a production per contraction of about 3.4 mgm. per cent. This figure corresponds nearly enough with the calculated lactic acid production attributable to the maximum contraction of an average work series, given above as 2.7 mgm. per cent, to lend support to the view stated on page 492 that the delay in acid production in mammalian muscles is less prolonged than in frog's muscles.

Fatigue. The conception that the force of contraction is determined (in general) by the amount of decomposition of phosphocreatine (p. 490) implies the further conception that decline in force of contraction, i.e., fatigue, is the result of a falling off in the amount of the same decomposition. Any factor which might operate to induce such a falling off thus becomes a potential cause of fatigue. The lactic acid theory of fatigue must obviously be tested from this point of view, as well as any alternative theories which suggest themselves.

One obvious alternative theory is that fatigue is due to accumulation of the decomposition products of phosphocreatine itself, an accumulation which may be conceived to reduce the volume of subsequent decompositions through a mass effect. The observation previously cited (p. 491) indicating a definite lag in resynthesis of phosphocreatine appears at first view to lend support to this idea, but the following considerations seem to us to throw grave doubt on its acceptability. According to the current picture of muscle metabolism, stimulating a muscle by a brief tetanus brings about an immediate explosive decomposition of some definite quantity of phosphocreatine, followed promptly by an onset of restorative processes, which may consist partly of reactions in which oxygen is utilized and partly of reactions in which production of lactic acid is the significant feature. The latter reactions, in turn, follow a course in which there is immediate production of a considerable fraction of the total amount of acid to be formed (in some cases not more than half; Hill, 1932, p. 63), followed by a declining acid production somewhat long drawn out, giving in the end, however, an energy-magnitude for the restorative process as a whole agreeing reasonably closely with the energy cost of resynthesizing the quantity of phosphocreatinine decomposed.

We cannot conceive how such a process as the explosive decomposition of a definite amount of an unstable compound from a considerable store of the same present in the muscle can of itself determine the magnitude and course of a subsequent restorative process such as we have under consideration here, but we can readily imagine that the immediate chemical result of the decomposition, namely, the accretion of a definite quantity of decomposition products, may so act. Proceeding on the latter basis we conceive, as the result of the sudden formation of some definite quantity of decomposition products of phosphocreatine, a definite series of reactions to be set in motion which almost immediately cause the recombination of a considerable portion into the parent compound, but leave a moiety to be recombined more gradually through the agency of that part of the lactic acid production which lags behind the rest.

Assuming a second brief tetanic stimulus to be applied shortly after the first the same sequence of events as described above is set in motion, but with the consequence that upon decomposition of the new quantity of phos-

phocreatine there is present a larger total quantity of decomposition products than at the corresponding stage following the first stimulus. Additional stimuli applied at the proper intervals accentuate the effect, giving thus the familiar phenomenon of summation. Our present interest is in the effect of this summation, not on force of contraction, but on the ensuing restorative processes. If we grant that the action of decomposition products in initiating and governing these restorative processes is susceptible of summation the processes themselves must also be thought of as summing in proportion. But the action of these restorative processes is to induce recombination of the decomposition products into phosphocreatine, hence to the extent to which they undergo summation they counteract the accumulation of the decomposition products themselves. As a matter of fact, as in other types of summation, a maximum will quickly be reached beyond which accumulation cannot continue. This is the significant point in our argument, for under any distribution of lactic acid production between the immediate phase and the delayed phase at all likely to occur in muscle, the maximum accumulation of decomposition products of phosphocreatine will be reached before fatigue phenomena begin to show themselves except perhaps to a very moderate extent.

Accumulation of lactic acid, on the other hand, will be particularly rapid during the period of summation and will continue thereafter as long as the energy-magnitude of the whole restorative process exceeds that of the oxidative phase, the only counteracting factor being the escape of lactates by diffusion into the blood. Our observation that lactic acid concentrations rise rapidly during the early stages of work periods (p. 496) is in harmony with this part of the conception. A contrary view, according to which decomposition products of phosphocreatine are conceived to accumulate, implies a correspondingly slower accretion of lactic acid, and is correspondingly less in harmony with the observed facts.

We conclude that the present trend of evidence, both our own and that offered by other investigators, tends to support the lactic acid theory of fatigue. We wish to make the point, however, that according to our observations the decline in developed tension which is interpreted as a manifestation of fatigue does not follow strictly the accumulation of lactates within the muscle. During the period of most rapid accumulation the tension developed in successive contractions remains nearly constant in many experiments. The suggestion is that there is some critical (and considerable) concentration of lactates which the fibers can endure without affecting the amount of phosphocreatine decomposition, but that after this critical concentration is exceeded further increases in concentration are accompanied by proportional or approximately proportional declines in the magnitude of the decomposition, and hence in the force of contraction.

The steady state. The above conception of fatigue leads directly to a con-

ception of the steady state as manifested in mammalian muscles in situ, according to which the state depends on the interaction of the following factors: 1, the decline in theoretical oxygen requirement as the force of contractions falls off; 2, the maintenance of the oxygen supply at its maximum, since the blood flow does not diminish; 3, the consequent decrease in production of excess lactic acid to a quantity which, at the diffusion gradient set up by the high concentration of lactic acid accumulated within the muscle, escapes by diffusion as fast as formed. When this situation is realized a metabolic equilibrium is established; no further accumulation of lactic acid takes place, and although no recovery is possible while the activity continues, neither is there a further decline in activity. It should be emphasized that this conception of the steady state implies it to be one of partial fatigue in which the active fibers are quite heavily charged with lactic acid.

Consequences of reduction in efficiency. The discussion thus far has proceeded on the assumption that in a given work period the tension developed in any contraction bears a fixed ratio to the quantity of phosphocreatine decomposed in the same contraction, although the possibility that under certain conditions the ratio may rise as fatigue advances is pointed out (p. 490). The tacit assumption has also been made that the energy cost of resynthesizing phosphocreatine is constant, although Meyerhof (1921) has shown that the "isometric coefficient of lactic acid," in other words, the ratio of phosphocreatine decomposition (as expressed in tension development) to the energy-magnitude of the restorative processes (expressed as lactic acid), falls considerably when fatigue becomes marked. These changes in ratio are in opposite directions and may conceivably counteract each other more or less completely in most instances. But we have some observations that seem to us to indicate that in certain cases the increased metabolic cost of resynthesizing phosphocreatine (a lowered isometric coefficient of lactic acid) considerably outweighs whatever increased economy of tension development may occur.

Whenever a muscle, under the conditions of our experiments, attains a steady state it does so, according to our interpretation, because the escape of lactates by diffusion moment by moment becomes equal to their production in the same intervals. This equality will obviously be reached sooner in a series of contractions in which the production of lactic acid decreases in strict proportion to the decline in tension development than in a similar series in which the production of acid per unit of developed tension rises. Even in the latter case, a steady state may be attained provided the change in the lactic acid coefficient is not too pronounced, but the level of tension development will obviously be lower. Or it may be that more lactic acid will come to be formed per unit of developed tension than can escape by diffusion at any stage, in which event lactates will continue to accumulate

until the muscle becomes wholly unable to contract. In the accompanying paper work periods are described which suggest the incidence of each of the three above described alternatives. All three are explicable in the main in accordance with our main conclusions, but by adding to the factors considered initially the further factor of fall in the lactic acid coefficient we arrive at an explanation of the phenomenon of muscular exhaustion, which is otherwise difficult to account for in the intact muscle *in situ*.

Consequences of partial activity. When the performance of the muscle is cut down as the result of the dropping out of the individual fibers *in toto*, either through myoneural junction "fatigue" or through failure of excitation of entire motor units, a somewhat different situation from that pictured thus far is presented. The inactive fibers are favored with the increased blood supply characteristic of the active muscle as a whole, but have a markedly reduced oxygen requirement, which must return nearly or quite to the basic level (some excess oxygen utilization may occur in metabolising the accumulated lactic acid). If the quiescent fibers are interspersed among active fibers the available oxygen supply to the latter is improved, their production of lactic acid is reduced to correspond, and the onset of fatigue is delayed in proportion. A steady state will presumably be established in course of time, but less rapidly in harmony with the less rapid onset of fatigue.

When the inactive fibers are in blocks, as is conceivably the case when entire motor units go out of action, or in the more pronounced situation in which the excitation is submaximal, leaving a considerable fraction of the muscle inactive, there will presumably be an increase in blood flow through the capillaries contiguous to these inactive fibers but too distant from active fibers for oxygen to penetrate from these vessels to them. The blood emerging from such capillaries into the veins must necessarily be less venous than the blood from the same vessels prior to the beginning of activity. Whether the effect can ever be so pronounced as to make the venous outflow from the muscle as a whole contain more oxygen during the work than prior to it is questionable. In the classical experiments of Chauveau and Kaufmann (1887) the blood from the resting muscle was invariably brighter than from the muscle in activity.

The metabolism of a muscle mass. With the above picture of the activity of individual muscles before us we turn to a consideration of the behavior of a large muscle mass exemplified in the experiments of our third group. As pointed out earlier (p. 490) the excess oxygen consumption per 100 grams of worked muscle per minute, calculated from the increased oxygen intake of the whole dog (table 5) is considerably larger than the corresponding figure determined directly on individual muscles *in situ* (4.7 cc. per 100 grams per minute during the first 3 minutes, as compared with about 1.8 cc. per minute during the 1.3 minute of the average experiment on single muscles).

This larger utilization of oxygen implies either a more copious blood flow or a greater arterio-venous oxygen difference. The weight of evidence indicates clearly, as already pointed out (p. 495), that the arterio-venous oxygen difference is not likely to be much greater than observed by us in these experiments, namely, about 13.5 volumes per cent. If we adopt this figure for the purpose of calculation, a blood flow of 42 ml. per 100 grams of muscle per minute must be postulated to permit of the utilization of 68 cc. of oxygen by 1200 grams of muscle per minute (4.7 cc. per 100 grams, plus 1 cc. per 100 grams basic oxygen consumption³). This is approximately twice the average flow through the worked gracilis muscle as given in table 3, but does not surpass the potential flow through a capillary bed widened to the maximum.

If we assume the blood flow to have been 42 ml. per 100 grams of muscle per minute the production and output of lactic acid in the experiments of this group can be estimated. The necessary additional data are given in table 4. They are strictly applicable only on the assumption that the diffusion of lactic acid from the active muscles into the venous blood is uniform moment by moment. But since the maximal production of acid occurs at the outset of activity, causing the muscles to become heavily charged with acid rather promptly (p. 496) the assumption has a sufficient degree of probability.

Forty-two milliliters of blood are thus presumed to have flowed through each 100 grams of active muscle per minute. According to the data of table 4 the average uptake of lactic acid by this quantity of blood was about 11 mgm. more than from resting muscle. In a work period of 15 minutes' duration each 100 grams of muscle is estimated, therefore, to have given off about 165 mgm. of lactic acid on the average. At the end of the work period each 100 grams of muscle still contained about 80 mgm. of lactic acid in excess of the quantity present in the resting muscle, indicating an average production of about 245 mgm. per 100 grams of muscle for the entire period of activity. It is clearly not legitimate to apportion this production of acid uniformly throughout the work period, since evidence has been presented painstakingly above to show that, on the contrary, the production is at its peak during the earliest stages of activity and declines thereafter to a considerably lower level. It happens that the average production of lactic acid per 100 grams of muscle per minute during work in the experiments summarized in table 3, agrees closely with the above figure for the excess acid remaining in the muscles of this series at the end of 15 minutes of activity, i.e., 80 mgm. If the amount estimated to have been taken up by the blood per minute, 11 mgm., be added to the above figure and the sum, 91 mgm., be presumed to represent the production of lactic

³ This basic figure is taken from table 3.

acid per 100 grams of muscle per minute during the first stages of activity the following metabolic data and estimates are in hand:

Excess oxygen consumption per 100 grams of active muscle per minute 4.7 cc.; per contraction 0.094 cc.; basic oxygen consumption per contraction 0.02 cc.; lactic acid production per 100 grams per minute 91 mgm.; per contraction 1.8 mgm.; oxygen equivalent of 1.8 mgm. lactic acid, 0.250 cc.; total average theoretical oxygen consumption per contraction 0.346 cc. This estimate exceeds the mean theoretical requirement as determined more precisely on single muscle (p. 493) by about 20 per cent, but in view of the uncertain character of some of the factors, notably the arterio-venous oxygen difference, which was not determined directly in these experiments, the average weight of muscle stimulated, which was computed from muscle weighings in only one dog, and the lactic acid production of the whole leg musculature, which was assumed to be at the same rate as in the gracilis, which was the only muscle analyzed, the agreement is probably as close as can be hoped for, and seems definitely significant as supporting in considerable measure the conclusions based on metabolic data from single muscles as presented in antecedent paragraphs.

SUMMARY

1. The partition coefficient for lactates between muscles and blood of dogs is found to be 2.

2. The assumption that in unfatigued muscle tension development is a reliable index of phosphocreatine decomposition is discussed and accepted as valid.

3. The resynthesis of phosphocreatine through the combined agency of oxygen utilization and lactic acid production is considered.

4. The conclusion is drawn that in mammalian muscles in typical work periods the delayed lactic acid production is so nearly completed during the periods themselves that immediately subsequent determinations of muscle lactates include the whole theoretical amount, both immediate and delayed.

5. The average energy requirement, expressed as the "total theoretical oxygen requirement" per 100 grams of dog's gracilis muscle per brief isometric tetanus is calculated from determinations of oxygen consumption and lactic acid production during rest and activity.

6. On account of limitations imposed by the capillary circulation only part of the theoretical oxygen requirement of the individual active fibers in an active muscle is actually met by oxygen. The remainder is met by excess production of lactic acid.

7. The greatest oxygen deficiency, and hence the greatest production of lactic acid, necessarily occur at or near the beginning of activity. This follows in part from the lag in widening of the capillary bed at the beginning of activity, and in part from the reduction in theoretical oxygen requirement attending the reduction in force of contraction as activity proceeds.

8. Lactates necessarily accumulate rapidly during the early stages of activity, setting up high diffusion gradients for lactates between muscles and blood.

9. The work here presented is interpreted as supporting the lactic acid theory of muscular fatigue. An alternative theory, that fatigue is due to accumulation of decomposition products of phosphocreatine, is discussed and found doubtful. Accepting fatigue as due to accumulated lactates the fact is brought out that fatigue does not develop in strict harmony with the accumulation of lactates within the muscle but becomes manifest only after a considerable concentration is present.

10. The "steady state" is considered to be dependent on the interaction of the following factors: *a*, the decline in theoretical oxygen requirement as the force of contraction lessens; *b*, the maintenance of the oxygen supply at its maximum, since the blood flow does not diminish; *c*, the consequent decrease in production of excess lactic acid to a quantity which escapes by diffusion as fast as formed.

11. If the "efficiency" of the muscle falls off during the later stages of a work period a steady state may still be established at a low level of tension development, provided the relative increase in lactic acid production does not cause the actual quantity formed to exceed the maximum that can escape by diffusion. Should the production exceed this maximum the contractile power necessarily declines to complete exhaustion.

12. In muscles active only in part the active fibers may obtain additional oxygen through the increased blood flow to adjacent inactive regions within the range of effective oxygen diffusion. Beyond this range the active fibers in a muscle contracting submaximally can derive no benefit from the increased blood flow through the muscle as a whole.

13. Data obtained from a muscle mass, consisting of all the main muscles of one hind leg, support the above conclusions based on studies of single muscles.

BIBLIOGRAPHY

- BARCROFT, J. AND T. KATO. 1916. *Phil. Trans. Roy. Soc.*, ccvii B, 149.
BRONK, D. W. 1930. *Journ. Physiol.*, lxix, 306.
CHAUVEAU, A. AND M. KAUFMANN. 1887. *Compt. rend. de l'Acad.*, civ, 1763.
DAVENPORT, H. A. AND H. K. DAVENPORT. 1928. *Journ. Biol. Chem.*, lxxvi, 651.
EGGLETON, P. AND M. G. EGGLETON. 1927. *Nature*, cxix, 194.
1928. *Journ. Physiol.*, lxv, 15.
EGGLETON, M. G. AND C. L. EVANS. 1930. *Journ. Physiol.*, lxx, 269.
FENG, T. P. 1931. *Proc. Roy. Soc. B*, cviii, 522.
FISKE, C. H. AND Y. SUBBAROW. 1927. *Science*, lxv, 401.
HARTREE, W. AND A. V. HILL. 1920. *Journ. Physiol.*, liv, 84.
1921. *Journ. Physiol.*, lv, 133.
HILL, A. V. 1926. *Muscular activity*. Baltimore.
1932. *Physiol. Rev.*, xii, 56.

- HIMWICH, H. E. AND W. B. CASTLE. 1927. *This Journal*, lxxxiii, 92.
HIMWICH, H. E. AND M. I. ROSE. 1929. *This Journal*, lxxxviii, 663.
HIMWICH, H. E., Y. D. KOSKOFF AND L. H. NAHUM. 1930. *Journ. Biol. Chem.*, lxxxv, 511.
LUNDGAARD, E. 1930. *Biochem. Zeitschr.*, ccxvii, 162.
MARTIN, E. G., J. FIELD, II AND V. E. HALL. 1929. *This Journal*, lxxxviii, 407.
1930a. *This Journal*, xciii, 672.
1930b. *Proc. Soc. Exp. Biol. and Med.*, xxviii, 162.
MARTIN, E. G., E. WOOLLEY AND M. MILLER. 1932. *This Journal*, c, 407.
MEYERHOF, O. 1921. *Pflüger's Arch.*, exci, 128.
SOMOGYI, M. 1931. *Journ. Biol. Chem.*, xc, 725.
VAN SLYKE, D. D. 1917. *Journ. Biol. Chem.*, xxxii, 455.
VERZAR, F. 1912. *Journ. Physiol.*, xlv, 243.

THE EFFECT ON OVULATION AND PREGNANCY OF BLOCKING THE PITUITARY CIRCULATION IN THE RABBIT¹

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Although Gley (1891) made attempts to ablate the pituitary in rabbits, the first complete removal of the hypophysis was probably performed by Fee and Parkes in 1929. Following partial decerebration they were able to ablate the gland and keep their animals alive for nearly 48 hours. From results obtained with this technique they stated that rabbits did not ovulate if the pituitary was removed within one hour after mating, and that ovulation did occur if the hypophysis remained intact for an hour or longer after mating.

Smith and White (1931) with an operative procedure which neither exposed nor injured the brain and which was followed by prolonged survival, secured ovulation in animals in which the pituitary was left intact for an hour and a quarter after mating, a somewhat shorter post-copulatory period followed by ovulation than was observed by Fee and Parkes. They were also able to confirm the findings of Deanesly, Fee and Parkes (1930) that corpus luteum formation takes place even though the pituitary is removed shortly after mating and consequently several hours before ovulation. They found, however, that although the corpus luteum appeared normal for the first two days of its development, the subsequent stages were atypical and that actual regression was evident after four days.

The procedure of hypophysectomy employed by Smith and White usually requires a minimum of an hour for the completion of the operation. It seemed desirable to shorten this time in order to secure more precise data concerning the actual time after mating necessary for the anterior pituitary to liberate enough hormone to cause ovulation, and at the same time not to subject the rabbits to decerebration so that they would remain healthy for long periods. The information secured by a new operative procedure in which this has been achieved and which has given additional information on the length of the post-copulatory period of secretion of the pituitary necessary for ovulation forms the first part of the paper.

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Fraenkel (1910), Hammond (1925), and others have shown that the ablation of the corpora lutea (or ovaries) terminates pregnancy in rabbits. The final proof that the corpus luteum hormone is essential for gestation in this form has been given by Corner and Allen. Since the development of the corpora lutea becomes atypical after two days following hypophysectomy (Smith and White, 1931), it seemed probable that the fully-formed bodies would also soon become non-functional in the absence of the pituitary secretion. Data were secured on this point by operating on pregnant rabbits. The results obtained form the second part of the paper.

OPERATIVE PROCEDURE. The technique of blocking the blood circulation of the pituitary involved all the preliminary steps used by Smith and White in ablating the gland. Their procedure of hypophysectomy will therefore be briefly described. The mouth is opened and the jaws firmly secured by a head clamp. The dorsal mucosa of the nose is exposed by an incision through the soft palate, the opening being enlarged by a small retractor. The ophthalmic veins lying in the nasal mucosa are compressed as they pass through the cavernous foramen (Krause, 1883). The capacious blood sinus in which the hypophysis lies is then packed with bone wax inserted through the foramen, the foramen enlarged with a dental reamer, and the pituitary capsule exposed. The capsule is then torn open and its contents removed with a cannula and negative pressure.

Since the pituitary is surrounded by the lake of blood contained in the cavernous sinus (more accurately the circular sinus formed by the cavernous sinuses and the connecting transverse sinuses) and is separated from the brain by a bony shelf and the diaphragma sellae, it seemed probable that if the sinus were completely occluded with bone wax, the pituitary would be somewhat compressed and would suffer from an interrupted blood supply without injury to the brain. The procedure is not time consuming and can be completed without interference with the pituitary up to the stage of injecting the wax and then at the precise time desired the blockage be effected. That the animals suffer no brain injury from this technique is indicated by their healthy condition and their long survival without nervous disorders. A few animals, however, did develop symptoms approximately a month after the operation and at autopsy it was found that a purulent exudate had filled the sella turcica, raising the diaphragma sellae and thus causing pressure on the brain. These infections were infrequent but could not be entirely prevented.

For control operations all the steps were complete up to the injection of the wax.

The effect on ovulation. The data on the effect of this procedure on ovulation are given in table 1. The pituitary circulation was blocked at various periods between 30 and 90 minutes after copulation and exploratory laparotomies performed 24 hours later. None of the four animals

operated on earlier than one hour ovulated. Only one of three ovulated when the procedure was completed one hour after mating. The two animals operated on one and one-quarter and one and one-half hours, respectively, after mating both ovulated, results which confirm the earlier findings.

In the control operations (table 1) in which the mucosa was removed from the edges of the foramen as in the other animals, but no wax packed into the sinus, the normal percentage of animals ovulated, even when the procedure was completed as early as twenty minutes after mating. This shows that an incision through the oral mucosa and the pressure applied to the nasal mucosa to occlude the veins do not affect ovulation. The only additional trauma suffered by the experimental animals is limited to the

TABLE 1
Data on ovulation

ANIMAL	TIME AFTER MATING	OPERATION	REMARKS
	<i>hours</i>		
R42	20	Control operation	6 ruptured follicles
R39	25	Control operation	No ovulation
R40	30	Control operation	8 ruptured follicles
R41	30	Control operation	7 ruptured follicles
R50	30	Cavernous sinus occluded	No ovulation
R27	35	Cavernous sinus occluded	No ovulation
R28	45	Cavernous sinus occluded	No ovulation
R36	45	Cavernous sinus occluded	No ovulation
R29	60	Cavernous sinus occluded	No ovulation
R37	60	Cavernous sinus occluded	7 ruptured follicles
R38	60	Cavernous sinus occluded	No ovulation
R30	75	Cavernous sinus occluded	6 ruptured follicles
R31	90	Cavernous sinus occluded	5 ruptured follicles

contents of the cavernous sinus which makes it highly probable that the pituitary, only, is injured. The data presented in the first table therefore demonstrate that the pituitary must liberate its secretions for at least a full hour after mating to cause ovulation in the rabbit.³

The effect on pregnancy. The certainty that the complete occlusion of the cavernous sinus prevents the liberation of the pituitary secretion and the small amount of trauma incidental to the occlusion makes the opera-

³ Since these experiments were completed two rabbits have been hypophysectomized within 50 minutes after copulation and one within 60 minutes. All three failed to ovulate. It has been previously shown that ovulation occurs if the pituitary remains intact for an hour and a quarter after mating. These data with the above experiments place the time necessary after copulation for the pituitary to remain intact to cause ovulation between 60 and 75 minutes.

tion of great value in investigations on the essentiality of the pituitary for the continuation of pregnancy. Fifteen pregnant animals were used, three for control procedures, seven for the occlusion of the sinus and five for the ablation of the pituitary. No operations were performed earlier than the seventeenth day of pregnancy since the fetuses are not readily palpable until about this time. The results are given in table 2. Abortion occurred in all of the experimental animals up to the 26th day of pregnancy, with the exception of R23 and R48, which died with badly macerated fetuses. Those operated on at the 26th and 28th days of pregnancy gave birth to both living and dead young. These deliveries

TABLE 2
Data on pregnant rabbits

ANIMAL	DAYS PREG- NANT AT OPERATION	OPERATION	REMARKS
R32	17	Ether 2 hours	Normal pregnancy
R47	17	Control operation	Normal pregnancy
R51	25	Control operation	Normal pregnancy
R48	17	Cavernous sinus occluded	Died on 4th day, retained fetuses
R26	17	Cavernous sinus occluded	Aborted on 3rd day
R43	25	Cavernous sinus occluded	Aborted on 3rd day
R45	26	Cavernous sinus occluded	3 live young born on 4th day
R49	26	Cavernous sinus occluded	Aborted on 2nd day
R44	28	Cavernous sinus occluded	6 living young born on 3rd day
R35	28	Cavernous sinus occluded	5 live and 3 dead young born on 2nd day
R25	17	Hypophysectomized	Aborted on 3rd and 4th days
R20	21	Hypophysectomized	Aborted on 2nd day
R23	24	Hypophysectomized	Died 3 days later, macerated fetuses
R22	25	Hypophysectomized*	Aborted on 3rd day
R64	27	Hypophysectomized	Aborted on 3rd day, fetuses dead

* Small fragment of pituitary found in this animal at autopsy.

can possibly be considered abortions, however, since in no case were the young suckled or otherwise cared for.

From these findings it may be stated that the termination of pregnancy is a constant result of blocking the pituitary circulation. That this is due to a pituitary failure seems highly probable since pregnancy was not terminated in the majority of the animals until approximately 72 hours after the operation. If trauma played a significant rôle, abortion within 24 to 48 hours might be expected. That blocking the pituitary circulation produces the same effect as ablating the gland is shown by the five hypophysectomized animals. The most interesting of these animals is R64

which gave birth to non-macerated fetuses on the 30th day of pregnancy, 3 days after total hypophysectomy, indicating that the posterior lobe is not essential for initiating uterine contractions in parturition. The constant results from both procedures indicates the profound effect on the pituitary caused by the complete occlusion of the sinus.

All of the animals subjected to the control procedures gave birth to normal litters which were suckled.

HISTOLOGICAL CHANGES AFTER BLOCKING THE PITUITARY CIRCULATION.

In part of the cases in which the cavernous sinus was occluded there was a complete necrosis of the entire pituitary with the exception of the stalk. The stalk, however, is not injured when complete hypophysectomy, as the term is generally applied, is performed. In other cases there was a fragment of normal hypophysis present. In animals with total destruction of the gland the histological findings were very similar to those described by Aschner (1912), Cushing (1912) and Ascoli and Legnani (1912), and others in the dog, and by Smith (1930) in the rat. Only very small follicles were present in the ovary. The uterus was of a castrate type and the vaginal mucosa was flattened. The zona reticularis of the adrenal had shown a fatty degeneration and the whole cortex was much diminished in width. The thyroids were subnormal in weight, and the follicular epithelium was much flatter than in normal animals. In animals with remnants of pituitary present, the histological picture was intermediate between the normal and those with complete degeneration of the gland. It therefore seems highly probable that the extent of the atrophy of the gland depends on the amount of wax packed into the sinus, which in turn determines the time necessary for a collateral circulation to be established. When the wax is packed in very firmly the ingrowth of new blood vessels is delayed until after the gland has become necrotic, while if the wax loosely fills the sinus it is possible for blood vessels to be reestablished before autolysis of the glandular tissue has taken place. For these reasons this technic is unsuited for experiments which last for some time or for those in which a restorative therapy is undertaken. For an experiment of this kind hypophysectomy is superior. However, for studies in which a rapid operation entailing a minimum of trauma is desirable and in which the pituitary needs not be permanently blocked out, the technic presented here is highly satisfactory.

DISCUSSION. Although Fee and Parkes (1929) stated that the pituitary must secrete for one hour following mating, in order for ovulation to take place in the rabbit, they gave no data in their results for the interval between 32 minutes and 1 hour and 30 minutes after copulation. One of the purposes of the present investigation was to determine more exactly the actual time required by the anterior pituitary to liberate a quantity of hormone sufficient to cause ovulation. Our results confirm the state-

ment of Fee and Parkes since only one of three animals ovulated when the pituitary was blocked 60 minutes after mating and none ovulated when the operation was performed earlier. Ovulation was not interfered with if the gland was left intact for a period longer than one hour.

Aschner (1912) reported that hypophysectomy resulted in abortion in dogs. Smith and White (1931) reported abortion in a 22 day pregnant rabbit, three days after hypophysectomy. Pencharz and Long (1931) working on rats found that the hypophysectomy on the 11th to 20th day of pregnancy resulted in a prolonged gestation followed by death of the mother. Smith (1932) demonstrated that the posterior lobe is not essential for fertilization or parturition in this species. In view of the work of Jones (1926) it appears not improbable that the failure of the rats of Pencharz and Long to undergo parturition is due to the decrease in the tonicity of the uterus. That such is the case also appears probable from the work of the latter in which they found that parturition occurred if a fragment of the anterior lobe remained in situ. Allan and Wiles (1932) have recently reported abortion in cats after hypophysectomy.

In rabbits pregnancy is terminated after hypophysectomy and after blocking the pituitary circulation. That this is due to a loss of the pituitary hormones and not to trauma is demonstrated by the control operations. Furthermore, since the work of Smith and White has shown that after the ablation of the pituitary the corpora lutea develop normally only for a short period and since the corpus luteum hormone is essential for the maintenance of pregnancy in the rabbit, it seems probable that abortion is induced by hypophysectomy indirectly through failure of the corpora lutea. The final proof, however, that such is the case will depend upon the maintenance of pregnancy after hypophysectomy by the injection of corpus luteum extract. That abortion in the animals with blocked pituitaries is not due to an excess secretion of the posterior lobe is demonstrated by the fact that the removal of both lobes of the pituitary terminates pregnancy.

The writer wishes to express his appreciation to Dr. P. E. Smith for his assistance in this study.

SUMMARY AND CONCLUSIONS

The time required for the anterior pituitary to liberate the minimum quantity of hormone necessary to induce ovulation in the rabbit after copulation is between one and one and a quarter hours.

The blocking of the pituitary circulation results in the termination of pregnancy in the rabbit. Identical results are produced by ablating the gland.

The technique followed in blocking the pituitary circulation is given and the histological changes in the various organs following this procedure are briefly described.

BIBLIOGRAPHY

- ALLAN, H. AND P. WILES. 1932. *Journ. Physiol.*, lxxv, 23.
- ALLAN, W. M. AND G. W. CORNER. 1930. *Proc. Exp. Biol. and Med.*, xxvii, 403.
- ASCHNER, B. 1912. *Pflüger's Arch.*, cxlvi, 1.
- ASCOLI, G. AND T. LEGNANI. 1912. *Münch. med. Wochenschr.*, 518.
- CUSHING, H. 1912. *The pituitary body and its disorders*. Philadelphia.
- DEANESLY, R., A. R. FEE AND A. S. PARKES. 1930. *Journ. Physiol.*, lxx, 38.
- FEE, A. R. AND A. S. PARKES. 1929. *Journ. Physiol.*, lxxvii, 383.
- FRAENKEL, L. 1910. *Arch. f. Gynec.*, xci, 705.
- GLEY, M. E. 1891. *Compt. rend. d. Soc. Biol.*, Sec. 9, iii, 843.
- HAMMOND, J. AND F. H. A. MARSHALL. 1925. *Reproduction in the rabbit*. London.
- JONES, E. W. 1926. *Anat. Rec.*, xxxii, 234.
- KRAUSE, W. 1884. *Anatomie des Kaninchens*. Leipzig.
- PENCHARZ, R. I. AND J. A. LONG. 1931. *Science*, lxxiv, 206.
- SMITH, P. E. 1930. *Amer. Journ. Anat.*, xlv, 205.
1932. *This Journal*, xcix, 345.
- SMITH, P. E. AND W. E. WHITE. 1931. *Journ. Amer. Med. Assoc.*, xcvi, 1861.

STUDIES IN ANAPHYLAXIS

I. THE APPEARANCE OF A PHYSIOLOGICALLY ACTIVE SUBSTANCE DURING ANAPHYLACTIC SHOCK¹

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There is considerable evidence that the sudden, profound fall in arterial blood pressure that characterizes acute anaphylactic shock in the dog is mainly due to reactions taking place in the liver. This was indicated by the early work of Manwaring (1910a, b) and has subsequently been substantiated by the work of a number of investigators.

In this early work Manwaring reported that by cross circulating a normal dog with a dog rendered anaphylactic, anaphylactic symptoms were provoked in the normal animal. This led him to conclude that vasodilator substances are set free in the circulating blood as a part of the anaphylactic reaction. These positive experiments of Manwaring were contradicted by Weil (1917a) who failed to find any evidence of vasodilator substances in the blood of dogs dying from anaphylactic shock. He withdrew blood from the carotid arteries of dogs dying in anaphylactic shock and introduced this blood intravenously into normal dogs with negative results. Weil and Eggleston (1917) also reported negative results with blood repeatedly perfused through the isolated anaphylactic liver. Consequently Weil (1917b) was led to conclude, even though definite chemical changes in the blood of dogs during anaphylaxis had been shown by the work of Jobling, that "transfusion experiments have revealed the fact that these chemical changes are without influence upon the development of the shock. They are simply the harmless by-products of the anaphylactic reaction of the sensitized liver, and as a matter of fact, accompany a variety of pharmacologic procedures which injure the liver, such as chloroform or phosphorus poisoning."

In 1925 Manwaring and his co-workers (Manwaring, Hosepian, O'Neill and Moy), although corroborating Weil's experiments that the transfusion into normal dogs of carotid blood drawn from dogs dying in anaphylactic

¹ A preliminary report of this paper was made before the Illinois Branch of the Society for Experimental Biology and Medicine, April 5, 1932 (Proc. Soc. Exper. Biol. and Med., 1932, xxix, p. 391).

shock was without definite effect, and apparently admitting that the earlier cross circulation experiments were not conclusive, nevertheless reported definitely positive results by transfusing hepatic vein blood of anaphylactic dogs into normal dogs. They also reported that the vascular anastomosis of a normal dog's hind quarters to an anaphylactic dog demonstrated typical though slight anaphylactic contractions in the urinary bladder and rectal stump of the normal animal. To the physiologically active substance or substances indicated by these experiments to be set free in the blood stream they gave the name of hepatic anaphylatoxin.

These positive and apparently conclusive experiments of Manwaring have prompted us to investigate the appearance and possible nature of such hepatic anaphylatoxins. Inasmuch as Manwaring's observations have not been universally accepted, we were desirous first of either confirming or denying the reported appearance of physiologically active substances during shock. As an indicator of such activity we chose the guinea-pig intestine because the presumptive evidence is very strong that at least one of the properties of a physiologically active substance having either a cause or effect relationship to the symptoms of anaphylactic shock should be that of contracting smooth muscle. In addition to looking for such activity in the blood we were interested in examining the thoracic duct lymph, since Calvary (1911) and Petersen and Levinson (1923) have noted a marked increase in the amount as well as marked changes in composition of the lymph during anaphylactic shock.

The dogs were sensitized by a single intravenous injection of from 1 to 8 cc. of horse serum or hog serum. In a few instances a simultaneous injection of a similar amount of serum was made subcutaneously. In four cases sheep serum was used as the antigen but as well marked anaphylactic shock did not develop in any of the four, it was discontinued. The relative ineffectiveness of sheep serum as a sensitizing antigen in the dog has been noted by previous workers. After an interval of fourteen days or longer, the dogs were anesthetized, cannulae inserted in the carotid artery and thoracic duct for recording blood pressure and collecting lymph respectively. The shocking dose of serum was injected into the femoral vein. During the course of ensuing shock, lymph was collected in small beakers, blood was withdrawn by syringe from the femoral vein and in some experiments from the portal vein and the inferior vena cava. For the latter procedure, the chest was rapidly opened and the needle inserted into the inferior vena cava about 2 cm. above the diaphragm. Although this method yielded hepatic vein blood diluted to a varying degree with other blood, it was considered desirable to avoid the trauma incident to methods permitting the collection of exclusively hepatic venous blood. If the shock lymph or blood samples showed any evidence of clotting, additional samples were collected to which heparin was added. The collected samples

of lymph and blood were tested for physiological activity by means of a strip of guinea-pig intestine suspended in a 200 cc. bath of aerated Tyrode's solution at 39°C. in the usual way.

An illustrative protocol is as follows:

Experiment 23. Female dog, 10 kilos.

January 29, 1932. 4 cc. horse serum, saphenous vein.

February 19, 1932. 1:00 p.m. Sodium barbital 0.275 gram per kilo intravenous.

Cannula in carotid for blood pressure. Cannula in thoracic duct.

2:30. 10 cc. blood withdrawn femoral vein, heparin.

2:45-3:00 p.m. Collection of normal lymph, 8 cc.

3:00. 10 cc. horse serum femoral vein. Marked fall in blood pressure after latent period of 40 seconds.

3:00-3:05. First sample of "shock lymph," 8 cc.

3:05-3:10. Second sample of "shock lymph," 10 cc.

3:10-3:20. Third sample of "shock lymph," 10 cc.

3:18. 10 cc. blood withdrawn from femoral vein. Dog apparently dying.

Chest rapidly opened and 50 cc. blood aspirated from inferior vena cava.

3:20. Dog dead.

Blood and lymph samples immediately tested on guinea-pig intestine.

10 cc. normal femoral vein blood plus heparin, no effect.

8 cc. normal lymph, no effect.

1 cc. first sample "shock lymph," definite contraction.

1 cc. second sample "shock lymph," definite contraction.

1 cc. third sample "shock lymph," very marked contraction.

$\frac{1}{2}$ cc. third sample "shock lymph," definite contraction.

10 cc. shock femoral vein blood, no effect.

1 cc. shock vena cava blood, slight contraction.

2 cc. shock vena cava blood, definite contraction.

5 cc. shock vena cava blood, marked contraction.

Tracings illustrative of the contractions obtained are shown in figures 1, 2, and 3 with legends which are self-explanatory. Attention is called to the fact that the contraction occurs immediately upon the addition to the bath of the active specimen of lymph or blood. This is particularly illustrated in figure 3 in which the small intestine of a sensitized guinea pig was used for the testing. Here a typical anaphylactic contraction with the usual latent period was first induced, and then after the intestinal strip had been thus desensitized, an active specimen of inferior vena cava blood produced an immediate contraction. Normal blood, drawn from either normal dogs, or from the sensitized dogs prior to shock and tested immediately or kept from clotting by heparin was consistently inactive even in doses of 25 cc. Active specimens of inferior vena cava blood obtained after shock, although incoagulable, did not lose any activity by the addition of heparin. Many samples of normal lymph were tested prior to clotting and were consistently inactive. Those prevented from clotting by heparin were likewise inactive. The addition of heparin to active specimens of shock lymph did not diminish their activity.

Most of the essential details of the experimental methods and results are indicated in the table. Attention is called to the method of grading the severity of the anaphylactic shock. Fatal shock is graded + + + +; non-fatal shock in which there is no appreciable recovery of the blood pressure from its low level within the period of one-half hour is graded

Fig 1

Exp 21 NORMAL PIG
 1 = 1cc Normal lymph
 2 & 3 = 1cc lymph 1st 5 min
 4 = 1cc lymph 2nd 5 min
 Hog serum Anaphylaxis + + + +

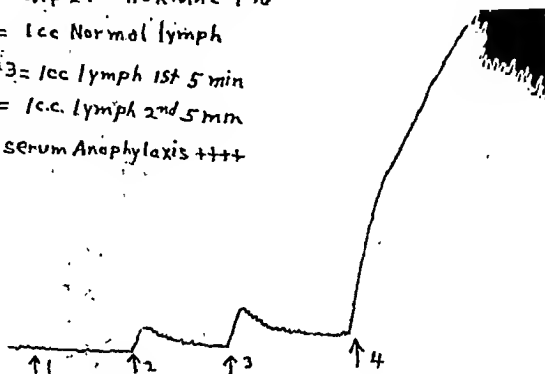


Fig 2.

EXP 23 NORMAL PIG

1 = 1cc inferior vena cava blood
 18 min. later 2 = 5cc do.
 Horse serum Anaphylaxis + + + +

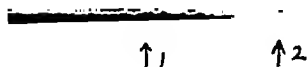


Fig 3

Exp 27 Hog Serum Sens Pig

1 & 2 = 1cc. Hog Serum
 3 = 10cc. inferior vena cava blood
 after hog serum anaphylaxis



+ + +; non-fatal shock in which the blood pressure has been markedly reduced but tends to recover within one-half hour is graded + +; and shock in which there is a negligible fall in blood pressure is graded +.

Thus it is seen that of twenty-seven cases of anaphylactic shock of varying degree in which the thoracic duct lymph was tested for physiological

activity on the guinea-pig intestine, the lymph was definitely positive in ten, questionably positive in two and negative in fifteen. However, of the eleven cases of severe or fatal shock, the lymph was positive in eight. Similarly the inferior vena cava blood was positive in eight experiments

TABLE 1

EX- PERI- MENT	KILOS	SENSI- TIZING DOSE OF SERUM	INTER- VAL	SHOCK DOSE	DEGREE OF SHOCK	SMOOTH MUSCLE STIMULATING PROPERTY OF				REMARKS
						Thoracic lymph	Inferior vena cava blood	Portal blood	Fe- moral blood	
		cc.	days	cc.		cc.	cc.	cc.	cc.	
3	8	1.25	21	3	++			-10	-10	Lymph tested after 48 hrs.
4	6.3	2	14	3	++++	+1				
5	6.3	2	15	3	++++	-5				
7	11	1½	15	3	++	+?5				
8	11	3	19	3	++	+?5	-10			
9	8.4	3	22	5	+++		+2			
10	7	3	23	5	+++	+1				
11	12.5	4.5	22	4	+	-5				
12	15	6	20	8	++++	+0.5				
13	12.7	5	27	5	++	-5				
14	16	5	20	7	++	-10				
15	19	8	22	7	+++	-10	-10			
16	10	5	30	10	++	-5				
17	17	5	16	10	++	-5				
18	12	3	16	10	++	+4				
19	11	3	19	7	++	-7	-10			
20	15.5	3	23	10	++	+0.5				
21	9.3	3	17	10	++++	+0.25	+4		-10	
22	11	3	25	10	++	-20				
23	10	4	21	10	++++	+0.25	+2		-10	
24	14	4	28	10	++++	+2	+5		-10	
25	19	4	21	10	+		-10		-10	
26	12	4	16	10	+	-5			-10	
27	12	4	21	10	++++	-6	+5		+10	
28	28	4	26	11	++	-10			-10	
31	11	4	45	11	++	-5				
35	17	3	25	10	++++	+1	+1	+10	-10	
36	11	3	27	10	++	-3	+5	-10		
37	26	4	36	10	++++	+5	-10	-20	-15	
38	15	3	18	10	++	-5	+25			

out of thirteen examined, while in fatal shock it was positive in five out of six. There is therefore some definite correlation between the severity of the shock and the appearance of the active substance. This correlation is not absolute as in the case of experiment 20, with a relatively mild degree

of shock, a highly active lymph was obtained, and in the case of experiment 27, with a fatal shock the lymph was inactive at least in doses of 6 cc. In most of the experiments in which both the inferior vena cava blood and thoracic duct lymph were examined there was a comparable degree of activity in both. However, in experiment 27 the blood was active and the lymph inactive (in doses of 6 cc.) while in experiment 37 the lymph was active and the blood inactive (in doses of 10 cc.). It is to be noted that the determination of physiological activity is expressed as positive or negative with respect to certain doses of lymph or blood added to the 200 cc. bath containing the intestinal strip, so that in some instances it is possible that a lymph or blood sample recorded negative in the specified amount might have been positive if further quantities had been available for testing. In every instance the irritability of the guinea pig intestine was checked by a standard solution of histamine. Therefore in most of the instances of recorded positive specimens of lymph or blood appearing in the table the accompanying quantity specified indicates that this amount provoked a contraction comparable to that produced by 0.001 mgm. of histamine dihydrochloride (e.g., in experiment 23, 0.25 cc. of lymph provoked a contraction equivalent to that of 0.001 mgm. histamine dihydrochloride and the activity of the lymph was approximately equivalent to that of a 1 to 250,000 solution of histamine dihydrochloride).

While not systematically studied in every experiment, considerable data were obtained with respect to the time relationship of the appearance of the active substance. Thus in several experiments in which adequate amounts of lymph were collected within three minutes from the time of injection of the shocking dose of serum to warrant testing, it was clearly evident that the activity was present at this early period. This was the case in all instances of fatal shock. In one case of non-fatal shock (++) the first five minute sample of thoracic duct lymph was negative in 3 cc. doses, while the second five minute sample was positive in 4 cc. doses. The time relationship was somewhat clearer in the case of experiment 12 (fatal shock). The first three minute sample was negative in 1 cc. doses, but positive in 5 cc., the sample collected between the third and sixth minute was positive in $\frac{1}{2}$ cc. doses, the sample collected between the sixth and fourteenth minute was positive in 1 cc. doses, and the sample collected between the fourteenth and twentieth minute was positive in 2 cc. doses. Here a definite rise and fall in the concentration of the active substance is indicated. In one experiment (expt. 9) in which the chest was kept open and the animal under artificial respiration, the inferior vena cava blood was positive three minutes after the shocking dose in amounts of 2 cc. and negative in doses of 10 cc. ten minutes later. In one case of non-fatal shock (expt. 20) the thoracic lymph was positive in $\frac{1}{2}$ to 1 cc. doses for thirty minutes after the shocking dose, then becoming negative in 4 to 10 cc. doses. It is apparent

therefore that the active substance appears in the thoracic duct lymph or inferior vena cava blood very promptly upon the onset of shock, and, if the shock is non-fatal, will decrease and disappear in a variable but comparatively short space of time.

Our experiments were not designed or performed to permit of any positive conclusions as to the source of the active substance appearing during shock. Our results are for the most part compatible with the conclusions of Man-¹wareing that the chief source is the liver. This is suggested for example by the observations in experiment 37 in which it was shown that the activity is present in the inferior vena cava blood but not in the portal vein blood. Similarly, as the evidence is quite strong that the increase in thoracic duct lymph occurring during anaphylactic shock is chiefly of hepatic origin, there is a considerable degree of probability to the inference that the active substance appearing in such shock lymph comes from the liver. On the basis of our experiments alone, however, other possibilities would probably have to be considered.

The almost regular absence of activity in the femoral vein blood would seem to indicate that the active substance disappears rapidly from the blood as it circulates. This is in harmony with the observations of Man-¹wareing and his co-workers who found that the transfusion of carotid blood from dogs dying in anaphylactic shock into normal dogs was essentially negative while the transfusion of hepatic vein blood produced definite anaphylactic symptoms. It is important to recognize, therefore, that this loss of activity on the part of the blood as it circulates may largely explain the negative results of numerous investigators who have failed to find physiologically active substances set free during anaphylactic shock.

During the course of the preceding discussion we have frequently referred to the blood or lymph specimens that had the property of stimulating the guinea-pig intestine as containing a physiologically active substance. That the acquisition of this activity by the blood or lymph is really due to an active substance is definitely shown by the experiments in the succeeding paper.

We do not propose to discuss at length what significance attaches to the appearance of a smooth muscle stimulating substance or substances in the inferior vena cava blood or thoracic duct lymph during anaphylactic shock in the dog. The proper interpretation will follow upon the elucidation of all the facts. Whether it represents a humoral toxic agent resulting from the anaphylactic reaction of antigen and antibody which serves to provoke some or all of the symptoms of anaphylactic shock or whether it is merely the harmless by-product of such a reaction is not demonstrated by these experiments. Or a third position may be taken, namely, that this active substance may indeed be identical with the toxic substance mediating some or all of the symptoms of shock, but that the amount which appears free

in the blood or lymph represents the excess production over and above that necessary to produce symptoms. As there is no distinct amelioration of shock produced by draining the thoracic duct lymph to the outside it is apparent that that portion of the active substance appearing in the lymph may be regarded as such an excess or at least a relatively unimportant fraction of that which is producing symptoms. It remains, however, to determine the nature and production of the active substance and the extent to which it may produce effects comparable to those appearing during shock.

CONCLUSIONS

1. In the majority of instances of severe or fatal anaphylactic shock in the dog there appears a substance or substances which have the property of stimulating smooth muscle (guinea-pig intestine) in *a*, the supra-diaphragmatic inferior vena cava blood, and in *b*, the thoracic duct lymph.

2. This substance apparently disappears or rapidly diminishes as the blood circulates as it can but rarely be demonstrated in the femoral vein blood.

BIBLIOGRAPHY

- CALVARY, M. 1911. Münch. Med. Wochenschr., xiii, 670.
MANWARING, W. H. 1910a. Johns Hopkins Hosp. Bull., xxi, 275.
1910b. Zeitschr. f. Immunität., viii, 1.
MANWARING, W. H., V. M. HOSEPIAN, F. I. O'NEILL, AND H. B. MOY. 1925. Journ. Immunol., x, 575.
PETERSEN, W. F., AND S. A. KEVINSON. 1923. Journ. Immunol., viii, 349.
WEIL, R. 1917a. Journ. Immunol., ii, 525.
1917b. Journ. Immunol., ii, 399.
WEIL, R. AND C. EGGLESTON. 1917. Journ. Immunol., ii, 571.

STUDIES IN ANAPHYLAXIS

II. THE NATURE OF A PHYSIOLOGICALLY ACTIVE SUBSTANCE APPEARING DURING ANAPHYLACTIC SHOCK¹

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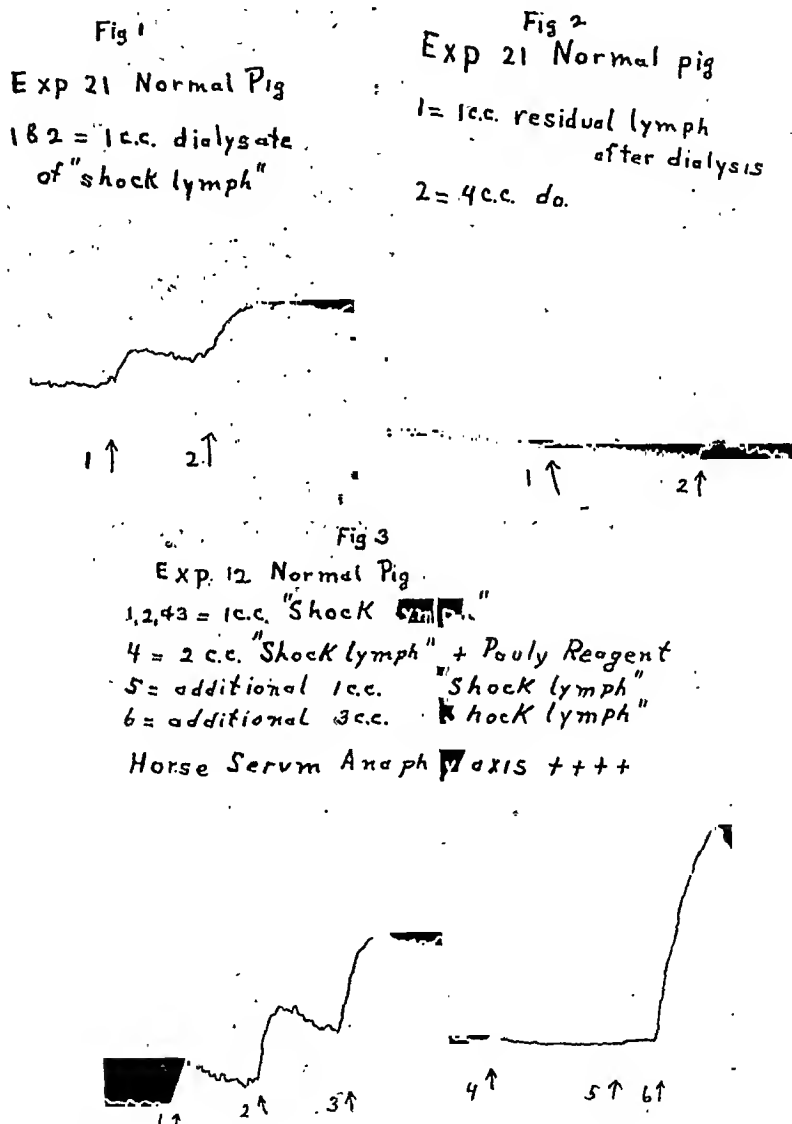
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It has been shown in the preceding paper that a physiologically active material may appear in the inferior vena cava blood and thoracic duct lymph of dogs during anaphylactic shock. In order to characterize the principle encountered a number of chemical and pharmacological experiments have been performed and are here reported.

1. The active material is dialyzable. The first question that presented itself was as to the molecular size of the active material. Dialysis of active blood and lymph samples was resorted to. Parchment sacs as well as cellophane tubing were used as semi-permeable membranes and the experiments carried out by dialyzing into an equal volume of either distilled water or 0.85 per cent sodium chloride for ten to twelve hours in the ice-box. In some instances a few drops of toluene were added to prevent bacterial growth, although even when not added no apparent bacterial growth took place. Care was taken to start the dialysis and transfer the experiment to the ice-box immediately after collection of the blood or lymph sample. When distilled water was used the dialysate was concentrated in vacuo to a small volume and the final concentrate adjusted to the proper pH for further testing. When normal saline was used the dialysate was not concentrated. The dialysates of active samples of blood or lymph were invariably active in stimulating the guinea-pig intestine (see fig. 1). The blood or lymph samples after dialysis retained little or none of the previous activity (see fig. 2). This experiment demonstrated the fact that the active substance is a crystalloid of relatively small molecular size. Recent work by Coca (1930) indicates that proteins ordinarily thought to be non-diffusible will pass through semi-permeable membranes in small quantities during the course of four or five days. However, the ease with which our material diffused leaves little doubt as to its molecular size.

¹ A preliminary report of this paper was made April 30, 1932 at the meeting of the American Physiological Society.

2. The active substance is predominantly basic. Having observed that the active substance dialyzed through semi-permeable membranes, the next step was to ascertain what type of compound it represented, whether acidic, basic or amphoteric. For this means electrodialysis was employed.



Active samples of shock blood or lymph were placed in the middle cell of a three-compartment electro-dialyzer. The two outer cells, fitted with platinum electrodes, were filled with distilled water or normal saline. Under an electric potential the activity of the middle cell rapidly diminished, while that of the basic cell increased correspondingly. The cathode

and anode compartments were cooled during the experiment. To prevent excess alkalinity which conceivably might destroy the active material, CO_2 was bubbled through the basic compartment. The middle cell was kept at approximately the original pH by appropriate additions of alkali. These experiments indicated that the physiologically active substance was either a base or predominantly basic in character at the pH of the electrodialysis. It has previously been shown (Gebauer-Fuelnegg and Kendall, 1931) that physiologically active bases can be separated from protein mixtures by electrodialysis.

3. The active substance is not specific. Since the active material has a small molecular weight we should not expect it to be immunologically specific. To confirm this view a series of guinea pigs were sensitized to and a second series immunized against hog or horse serum respectively. Active specimens of blood and lymph were obtained from dogs during anaphylactic shock induced by either hog or horse serum. Such active samples were equally active in stimulating the intestine of guinea pigs whether normal or sensitized to, or immunized against the corresponding serum. Similarly the blood or lymph from hog serum anaphylactic dogs was equally active when tested on horse serum sensitive or horse serum immune guinea pigs and vice versa the blood or lymph from horse serum anaphylactic dogs was equally active on hog serum sensitive or hog serum immune pigs. For the most part the immune guinea pigs had a titer of from 1 to 500 to 1 to 1000. As was pointed out in the preceding paper the contraction of the guinea pig intestinal strip induced by an active shock blood or lymph sample is an immediate one and does not have the latent period so characteristic of an anaphylactic contraction induced by the addition of the antigen to an intestinal strip of a sensitized animal. On the basis of these findings we conclude that the active substance is identical irrespective of the antigen used and definitely lacking in any specificity. This is contrary to the view expressed by Manwaring, Marino, McCleave and Boone (1927).

4. The active substance is inactivated by diazotized sulphanilic acid. In the course of the aforementioned experiments it was noticed that diazotized sulphanilic acid will inactivate the active substance in the presence of an excess of sodium carbonate. Solutions of the red condensation product (Pauly's reaction) failed to contract the guinea pig intestine after neutralization of the excess sodium carbonate. (See fig. 3.) We know that only a limited number of naturally occurring amino acids or amines will give condensation products with diazotized aromatic amines or their derivatives: tyrosine and tyramine as well as histidine and histamine. In addition protein split products of higher molecular weight such as peptides, etc., containing these amines or amino acids will also give a positive Pauly reaction. The inactivation by diazotized sulphanilic acid

seems a very important indication that the active material belongs to the group of compounds just mentioned. From this group we can exclude from consideration the two amino acids and probably all peptides except possibly those containing histamine or tyramine. With respect to histamine it has recently been shown (Gebauer-Fuelnegg, 1932) that under proper conditions two mols of diazotized sulphanilic acid will effectively inactivate one mol of the base. It is realized, however, that a physio-

Fig 4

Exp 21
Cat-2 Kilo Ether 0.2 mg Atropine
1-4.5 c.c. dialysate from
"Shock lymph"

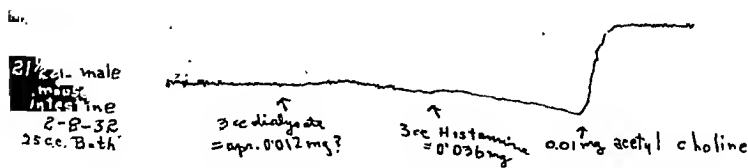


1

Fig 5

Exp 21

"Shock lymph" dialysate - no effect on mouse intestine



logically active substance hitherto unknown might exist that gives a positive Pauly reaction.

5. The active substance causes a fall in blood pressure in the etherized, atropinized cat.

Since the reactions of the active substance reported above were in accord with the assumption that it may be histamine, a number of further tests were made that are more or less typical for this substance. A dialysate

from an active shock lymph, part of which was first assayed on the guinea-pig intestine was injected intravenously into an etherized and atropinized cat. A prompt, temporary fall in blood pressure resulted. See figure 4.

The assay of the dialysate on the guinea-pig intestine expressed as histamine equivalent was closely approximate with that of the assay on the cat when similarly expressed. Inasmuch as the depressor response in the cat is considered by many as definitely characteristic of histamine and there is good quantitative agreement between the two methods of biological assay on the assumption that the active substance is histamine considerable support to such an assumption is afforded by these experiments.

6. The active substance produces characteristic wheals. Dialysates of active lymph and blood samples were found to produce characteristic wheals when endermally inoculated in human skin. While this reaction is by no means specific, it is consistently found with histamine solutions of adequate concentrations. Control wheals were produced with histamine solutions of known concentration and the values obtained by the wheal test were in harmony with those by the preceding methods of assay.

7. The active substance does not contract the mouse intestine. It has recently been reported (Wedum and Gebauer-Fuelnegg, 1932) that the intestine of the mouse is insensitive to histamine while highly reactive to acetyl choline. Dialysates of active specimens of blood or lymph, highly active on the guinea pig intestine, were without effect on the mouse intestine. See figure 5. This, as do a number of the preceding experiments, definitely excludes acetyl choline from consideration.

8. Miscellaneous.

Active specimens of lymph or blood retain their activity for at least one week in the ice-box, but lose their potency if kept in the incubator for twenty-four hours. Dialysates of active lymph or blood, brought to pH 7.6, have their activity diminished by prolonged drying on the water bath. Slightly acidified dialysates are stable to boiling. Specimens of normal blood and lymph to which histamine was added in comparable amounts gave similar results when carried through the above procedures. The contraction of the guinea-pig intestine induced by an active specimen of blood or lymph or their dialysates, is relaxed by the addition of formaldehyde. It has been shown that this is also the case in contractions induced by histamine and the anaphylactic reaction. (Kendall and Varney, 1927.)

SUMMARY. Summarizing the evidence presented in the preceding sections, it may be stated that the active substance occurring in the inferior vena cava blood or thoracic duct lymph of dogs during anaphylactic shock, has the following characteristics: It is a dialyzable crystalloid of basic

properties. It stimulates the smooth muscle of the guinea-pig intestine obtained from pigs that are either normal or sensitive or immune to either the homologous or a heterologous serum. The activity is associated with substances giving the Pauly reaction as it is inactivated by condensation with diazotized sulphanilic acid. It depresses the blood pressure of the etherized, atropinized cat but does not contract the smooth muscle of the mouse intestine. It produces characteristic wheals in human skin. Acid solutions are stable to boiling, while neutral or slightly alkaline solutions are somewhat unstable to heat. All of the above criteria are in accord with the conclusion that the active substance is histamine. This conclusion is strengthened by the fact that if histamine in adequate concentration is added to normal blood or lymph samples and these then carried through the same procedure similar results are obtained. In addition there is quantitative agreement between histamine solutions and the active substance when tested by three widely different methods of biological assay.

Although the above evidence would seem to be conclusive, it would be desirable to isolate histamine in crystalline form or to include spectroscopic evidence of its presence. However, the quantities of histamine indicated by our studies to be present are not such as to warrant the hope that either method would be satisfactory. For example, the maximal amount of active lymph obtained in a single experiment was 50 cc. and the maximal amount of inferior vena cava blood 200 cc. Assuming the average activity to be 0.001 mgm. histamine dihydrochloride per cubic centimeter of lymph and 0.0005 mgm. per cubic centimeter of blood, we are dealing with a total of 0.15 mgm. dihydrochloride or approximately 0.09 mgm. of base.

In the experiments where more active samples of lymph or blood were obtained the volumes were generally smaller owing to the rapid death of the animal. Data soon to be published indicate that spectroscopic assays of histamine are not delicate enough to determine these small quantities. Pooling the samples of several experiments has not yet been done.

Experiments on the inactivation of the active substance by the specific enzyme histaminase described by Best and McHenry (1930) are now in progress.

As to the origin of the histamine here shown to be present in the inferior vena cava blood and thoracic duct lymph during anaphylactic shock, there are a number of possibilities to be considered. Among them may be mentioned 1, the possibility that histamine originates from the blood; 2, that it is of cellular origin; 3, that it comes from the gastro-intestinal tract, and 4, that it comes from the liver secondary to the engorgement resulting from the shock. A discussion of these points is contemplated for the near future.

CONCLUSION

The active substance shown in the preceding paper to appear in the inferior vena cava blood and thoracic duct lymph during anaphylactic shock in dogs is apparently histamine.

Upon the completion of this paper our attention was called to a paper by Bartosch, Feldberg and Nagel in Pflüger's Archives of May 24, 1932. These authors report in a very interesting paper perfusion experiments on the lungs of anaphylactic guinea pigs demonstrating the occurrence of a physiologically active substance in the perfusates. They concluded that this substance was histamine. This is a valuable confirmation of our experimental findings from an entirely different method of study.

BIBLIOGRAPHY

- BEST, C. H. AND E. W. MCHENRY. 1930. Journ. Physiol., lxx, 349.
COCA, A. F. 1930. Journ. Immunol., xix, 405.
GEBAUER-FUELNEGG, E. 1932. Proc. Soc. Exper. Biol. and Med., in press.
GEBAUER-FUELNEGG, E. AND A. I. KENDALL. 1931. Beitr. d. Deutsch. Chem. Gesell., lxiv, 1067.
KENDALL, A. I. AND P. L. VARNEY. 1927. Journ. Inf. Dis., xli, 143, 156.
MANWARING, W. H., H. D. MARINE, T. C. MCCLEAVE AND T. H. BOONE. 1927. Proc. Soc. Exper. Biol. and Med., xxiv, 553.
WEDUM, A. G. AND E. GEBAUER-FUELNEGG. 1932. Proc. Soc. Exper. Biol. and Med., xxix, 888.

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THE INTRAPERITONEAL ADMINISTRATION OF VIOSTEROL IN MICE

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The aim of this study was to determine if irradiated ergosterol² is efficacious when injected into the peritoneal cavity.

During the course of experiments dealing with the calcification of the Twort mouse carcinoma (in vivo) by means of viosterol (7), the animals receiving medication frequently had diarrhea. It was presumed that such an abnormal condition of the gastro-intestinal tract might have not only an adverse effect upon the health of the mice but might well lead to a non-utilization of much of the viosterol. Therefore, the present investigators were confronted with the finding of a parenteral route by which the activated ergosterol might act efficiently and yet cause no effects detrimental to the object of the investigation. The decision to inject the substance into the peritoneal cavity was influenced by the report of Koehne and Mendel (1) which stated that the Vitamin D principle in cod liver oil afforded some protection to rats when given in this manner, and our enthusiasm was kindled somewhat by the fact that (at that time) we found no articles dealing with this mode of administration for viosterol.

EXPERIMENTAL MATERIALS AND METHODS. The irradiated ergosterol, as received from the manufacturers, was concentrated in sesame oil to a biologically-assayed strength of 10,000 times the antirachitic potency of cod liver oil (10,000 CLO). In order to obtain solutions of 1,000 CLO and 2,000 CLO, which were used in certain phases of the present investigation,

¹ Submitted to the Faculty of the Yale School of Medicine as partial requirement for the degree of Doctor of Medicine.

² The viosterol was obtained through the courtesy of Mead Johnson & Co., Evansville, Indiana.

Such terms as viosterol, irradiated ergosterol, and activated ergosterol are used interchangeably throughout the present article.

proper dilutions of the 10,000 CLO preparation were secured by the addition of sesame oil, which was supplied by the manufacturers for this purpose.

The mice were adults of both sexes and in some instances were the same as those used for the above mentioned experiment concerning the calcification of a mouse tumor. The diet consisted of oats and lettuce. In the first experiment milk was given, but it was omitted in the later ones. All had free access to tap water. Only apparently healthy animals were introduced into the investigation, and careful attention was given to ventilation, light, and the weekly sterilization of cages. Despite these precautions, the colony was depleted several times by epidemics of mouse typhoid. However, this condition was finally overcome, and the data from affected animals were discarded.

Reasonable cleanliness was observed, but it was not found necessary to disinfect the skin or even to sterilize repeatedly the needle and syringe. Neither before nor at the time of necropsy was an infection ever noted. The sesame oil, containing the irradiated ergosterol, was slightly warmed in order to secure a uniform solution.

With the back of the mouse grasped in the usual manner and the abdomen turned uppermost, the desired amount of sesame oil was injected into the peritoneal cavity. The procedure proved to be extremely simple and reliable as determined by immediate and subsequent celiotomies. One person easily accomplished it, and many mice were administered to in a short time.

The silver nitrate method of Von Kossa was employed for the histologic identification of calcium. The counter stain was alum carmine. In certain instances hematoxylin and eosin were resorted to for the study of tissue structure. Seventy per cent ethyl alcohol was quite satisfactory as a fixing agent and was more convenient than formalin on account of having to keep the latter neutralized in order to prevent loss of calcium from the tissues due to an acid medium.

The daily, general condition of the animals was noted. Some were allowed to die. Others were killed when it became apparent that they would survive only a short time. Thus we had at our disposal tissues which were fresh and also those which had been subject to postmortem autolysis for a few hours. As would be expected, in the former instance the results were ordinarily more satisfactory as regarded the detection of calcium as well as the preservation of the normal histology.

GENERAL PLAN OF THE EXPERIMENTS. After preliminary investigations had been carried out, further studies were conducted, of which the two reported below may serve as examples.

In one of these inquiries, designated as experiment 1, a total of 27 mice was employed. Eleven of the 27 were given viosterol of 1,000 CLO (the

original 10,000 CLO having been diluted with sesame oil) over a period varying in the individual animal from 25 to 61 days. In the same experiment a second group, consisting of 9 mice, received equivalent amounts of sesame oil (which contained no activated ergosterol) during a time interval of 22 to 61 days. The third and final lot of experiment 1 contained 7 individuals, which had nothing but the regular diet and were sacrificed at intervals varying from 26 to 61 days.

In experiment 1 a total of 17 intraperitoneal injections of a sesame oil solution of viosterol (1,000 CLO) was given. The first 12 doses each contained 0.1 cc., which was increased to 0.133 cc. in each of the last 5 administrations. In each of the animals which survived the period of 61 days, the entire amount received by any one individual was 1.865 cc., and the average dose was 0.11 cc.

In experiment 2 a total of 35 mice was utilized. Twenty-four of these were given sesame oil solutions of viosterol (2,000 CLO and 10,000 CLO) over a period varying in the individual animal from 43 to 52 days. A second group in experiment 2 contained 11 mice, the members of which had only the regular diet and were sacrificed in from 43 to 51 days.

In the first 10 injections of experiment 2, a sesame oil solution of viosterol (2,000 CLO) was employed. In each mouse the dose of the oil varied from 0.048 cc. to 0.160 cc. with an average of 0.0832 cc. and a corresponding total of 0.832 cc. In the same experiment (no. 2), the original undiluted sesame oil solution of viosterol (10,000 CLO) was utilized in the last 9 injections, in this instance the amount varying in each mouse from 0.016 cc. to 0.128 cc. with an average of 0.094 cc. and a total of 0.846 cc. When the above values from the two divisions of experiment 2 are translated into terms of 1,000 CLO, the total dose received by each mouse in experiment 2 would have been equal in antirachitic potency to 10.124 cc. of a sesame oil solution of viosterol as used in experiment 1, which, in the same terms, would amount to an average injection of approximately 0.533 cc. for each of the 19 doses administered in experiment 2. Thus it will be seen that the mice in experiment 2 were given much greater relative amounts of activated ergosterol than those in experiment 1.

In order to arrive at a correct evaluation of the results produced by the viosterol, it was endeavored to control any effects which might have been caused by the sesame oil (p. 529, expt. 1, group 2).

RESULTS. After about 30 days, depending somewhat upon the dosage employed, the mice exhibited anorexia followed by loss of weight. Continuation of the activated ergosterol resulted in bodily inactivity and ruffling of the hair. However, up to this point the cessation of viosterol administration was followed by apparent complete recovery of the animals. In the last stages of the illness there appeared extreme unsteadiness of movements, and the tails were cyanotic. From this point, even if medica-

tion were discontinued, the disease progressed steadily and rapidly until death.

In each mouse, which lived more than 30 days after receiving viosterol, abnormal calcium deposits were seen in various organs as revealed by the Von Kossa histologic method. This may be expressed in figures by stating

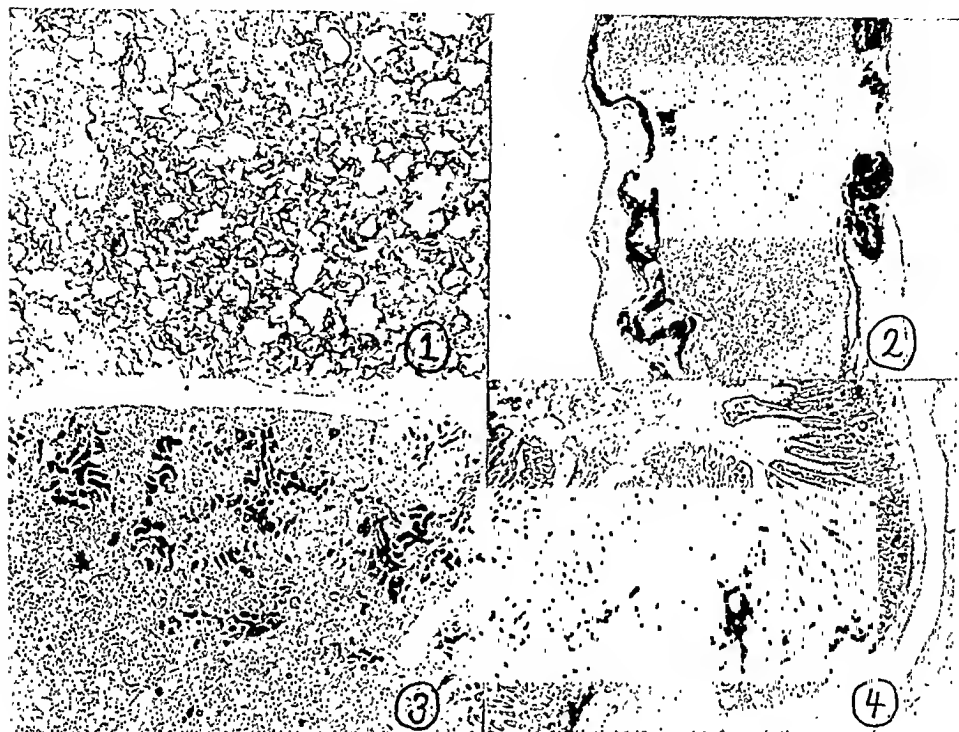


Fig. 1. $\times 45$ —Lung. Von Kossa stain. Calcium appears as black areas in the alveolar septa and in a large arteriole (left border of photograph).

Fig. 2. $\times 45$ —Aorta. Von Kossa stain. The black portions of the intima and media represent large calcium deposits.

Fig. 3. $\times 45$ —Kidney. Von Kossa stain. Many tubules are completely replaced by calcium, which shows as very black spots. Involvement of blood vessels is not seen in this part of the section but was noted in other portions. A partially calcified glomerulus may be observed near the left border of the photogram.

Fig. 4. $\times 25$ —Stomach and duodenum. Von Kossa stain. The black areas represent calcium. The pyloric muscle (projection in lower center) is seen separating the stomach on the left from the duodenum on the right. Calcification is evident in both organs.

that out of 35 animals which received activated ergosterol, there were 29 that exhibited pronounced calcification of certain viscera (figs. 1, 2, 3, and 4). The remaining 6 mice presumably lived too short a time (less than 30 days) for the lesions to become demonstrable.

We noted no calcific processes in the vena cava, esophagus, liver, pan-

creas, adrenal gland, ovary, testis, or cecum. In the *heart* and *spleen* the calcium deposits were limited to the arteries. In the *lung* calcium was found in the bronchial cartilages and mucosa, alveolar walls, and arteries. In the *aorta* very extensive calcification was seen in the intima and adjacent portion of the media. Calcific changes in the aorta appeared less often than in the stomach, lungs and kidney; but, when present, they were very marked. In the *kidney* calcium was deposited within the cells and the lumina of convoluted tubules, the intertubular spaces, arteries, and infrequently in the mucosa of the pelvis. In the *stomach* the cells of the glands, the muscularis mucosa, and the arteries of the submucosa were constantly and markedly affected. Pronounced calcification was occasionally seen in the prepyloric portion of the stomach, a finding contrary to that of another observer (3).

In no instance did the sesame oil per se have any apparent influence upon the deposition of calcium in the various organs.

DISCUSSION. It may be seen that in the main we have confirmed the observations of other workers as to the deposition of calcium by means of viosterol. In so doing, *the efficacy of the intraperitoneal route of administration has been established.*

However, we differ from Kreitmair and Moll (2) in that we did not find deposits of calcium in the muscles and adrenals, and from Smith and Elvove (5) in that we observed calcification of the pulmonary and renal arterioles, thus in the case of the kidney supporting the findings of Spies and Glover (6), and differing from Laas (3) in noting calcific changes in the prepyloric portion of the stomach and in the duodenum. It is quite possible that certain differences as observed by various workers may have resulted from diversity in the susceptibility of animals and in the duration of therapy, or in the amount and potency of the viosterol employed, as well as the routes and methods of administration.

A doubt as to the efficacy of the intraperitoneal injections of viosterol might arise by assuming that at times the needle entered the gut, thereby depositing the substance within the gastro-intestinal tract, and that the results which we secured may have been obtained by absorption through this route rather than by way of the peritoneum. It is not likely that such accidents could have happened sufficiently often to have seriously influenced our results. It will be remembered that our vehicle for the irradiated ergosterol was sesame oil. In no instance did we ever find the latter within the intestines, and we were always able to demonstrate it in the peritoneal cavity soon after injection and also after a lapse of several days. Furthermore, no infection was noticed, a thing which might have happened by even a small puncture of the gut.

It would appear, therefore, that the injection of activated ergosterol into the peritoneal cavities of mice has proven to be easy, reliable, and safe.

As this work was drawing to a close, Reed and Thacker (4) reported that viosterol was well utilized by dogs in whom it had been injected into the peritoneal cavity. Thus our experience in mice confirms theirs in dogs. However, they stated that when once the toxic symptoms of overdosage occurred they progressed, and death inevitably resulted. Often when our animals evidenced profound toxicity, as indicated by ruffled hair, anorexia, and disinclination to activity, we were able to restore them to normalcy by omitting the medication for a time. This difference as to recovery may have been due to proportionate variations in single dosages; for, when once the substance was injected into the peritoneal cavity, it was not removed, and a large amount of the sesame oil might have contained sufficient viosterol for the prolongation of toxic effects, finally terminating in the death of the animal whether more injections were made or not. This view is supported by the statement of Smith and Elvove (5) to the effect that the toxicity of activated ergosterol was related to the size of the single dose rather than to the total amount given over a certain period.

Reed and Thacker (4) reported calcification in the kidneys of their dogs, but they did not describe the technic employed for the histologic identification, and they apparently placed no emphasis upon the finding.

Since vitamin D is absorbed via the peritoneum, it may be that other fat soluble substances are capable of like action. Koehne and Mendel (1) have shown that this is true to a certain extent for vitamin A.

We have made no attempt to investigate the effects of the sesame oil and cannot therefore give any data concerning it except that it was apparently absorbed to a partial degree and did not cause obvious consequences in the animals.

It is our belief that the intraperitoneal method is applicable for laboratory animals, where the utilization of an exact amount of viosterol is desirable and where the avoidance of diarrhea is advisable.

Also, we wish to advocate the use of this technic, or a modification, in studies concerned with the way in which vitamin D acts, for it is now thought by many that its function is to increase the absorption of calcium from the intestinal tract. If this be true, then vitamin D which has been introduced parenterally, must be excreted into the gut or else be able to elaborate changes in the intestinal epithelium either by its actual presence there or by the action of secondarily evolved substances.

SUMMARY. The purpose of this study was to determine if viosterol could act efficaciously when injected into the peritoneal cavity of mice. The problem arose in connection with another which required the utilization of large, definite amounts of irradiated ergosterol (7) and the avoidance of diarrhea, which in our experience frequently accompanied the gastric and stomatic routes of administration.

A simple, safe, and reliable method has been described for the intraperitoneal administration of viosterol in mice.

The general condition of the animals and the histologic identification of calcium were the criteria employed for the evaluation of effects.

Two experiments have been reported, comprising a total of 62 mice, 35 of which were given viosterol intraperitoneally. In addition, 18 normal animals received nothing but the routine laboratory diet. Nine other mice were run as controls for any effects that might be produced per se by the sesame oil, since this was used as a vehicle for the viosterol.

The animals which lived more than 30 days (29 mice out of a total of 35) after the beginning of the viosterol injections revealed calcification in various organs, being most marked in the lung, aorta, kidneys, and stomach. These findings have been briefly compared with those of workers using other modes of administration, and the marked similarity of results indicates that the action of viosterol by way of the peritoneum is the same as by other routes in so far as the general condition of the animals and the deposition of calcium are concerned.

The possibility of the needle occasionally piercing the gut has been discussed with the conclusion that such was unlikely, and that infrequent mishaps of that nature could not have accounted for the marked effects which were uniformly obtained.

In the discussion we have pointed out certain applications that might be made of parenteral routes for the administration of fats and oil, including substances solvent in them.

CONCLUSIONS

1. The intraperitoneal injection of viosterol in mice has been established as a safe, simple, and reliable method of administration.
2. Calcium deposits were most marked in the lungs, stomach, kidney, and aorta.
3. The sesame oil (vehicle for the viosterol) did not have any demonstrable influence upon the calcific processes.

BIBLIOGRAPHY

- (1) KOEHNE, M. AND L. B. MENDEL. *Journ. Nutr.*, 1929, i, 399.
- (2) KREITMAIR, H. AND T. MOLL. *München. med. Wochenschr.*, 1928, lxxv, 637.
- (3) LAAS, E. *Virchow's Arch. f. Path. Anat.*, 1930, cclxxviii, 346.
- (4) REED, C. I. AND E. A. THACKER. *This Journal*, 1931, xvi, 21.
- (5) SMITH, M. I. AND E. ELVOVE. *U. S. Public Health Repts.*, 1929, xlv, 1245.
- (6) SPIES, T. D. AND E. C. GLOVER. *Amer. Journ. Path.*, 1930, vi, 485.
- (7) SPIES, J. W. AND G. P. LYMAN. Unpublished studies.

THE EXCRETION OF URINE IN THE DOG

VI. THE FILTRATION AND SECRETION OF EXOGENOUS CREATININE

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The excretion of ingested creatinine was recommended by Rehberg (1926) as a measure of glomerular filtration on the grounds that this substance is concentrated by the kidney to a greater extent than any other substance in the urine. Behre and Benedict (1922) had previously expressed doubts about the existence of creatinine in normal blood in sufficient quantities to account for the creatinine normally excreted, but Rehberg concluded that, since ingested creatinine is excreted by the kidney with the same readiness as the chromogenic substance or substances which are normally present in the blood, the main part of this chromogenic substance must be really creatinine.

Behre and Benedict's conclusion that part of the chromogenic substance in the blood is not creatinine has been confirmed by Gaebler and Keltch (1928), by Gaebler (1930) and by experiments of ours reported in this paper. Meanwhile Marshall and Grafflin (1928, 1930, 1932) have shown that exogenous creatinine is secreted by the aglomerular fish kidney, and also by the glomerular fish kidney both when the glomeruli are functional and when they have been rendered non-functional by the irritant action of phlorizin; and Clark and Smith (1932) have shown that exogenous creatinine is secreted by the elasmobranch kidney, although it is now well established that this substance is not normally present in significant amounts in the urine of these or other fishes.

These facts render the use of creatinine as a measure of glomerular filtration in the mammal suspect, and it is of interest, therefore, to compare the excretion of creatinine with the excretion of the non-metabolized sugars which Jolliffe, Shannon and Smith (1932) have recommended for this purpose.

The sugar methods used here have been fully described in the paper referred to above, and only a brief comment will be added here. For the determination of creatinine at low plasma levels, we have endeavored to devise a method which will distinguish true creatinine from other chromogenic substances present in plasma and urine. This method is described

in an appendix to this paper. By this method at least three substances, or groups of substances, can be distinguished in plasma:

1. Creatinine (of either exogenous or endogenous origin; in this category there will also fall the substituted creatinines which behave toward Lloyd's reagent like creatinine).¹

2. Non-creatinine chromogenic substances which are distinguishable from creatinine by the fact that they are not absorbed by Lloyd's reagent.

3. A non-chromogenic substance (other than creatinine or creatine) which yields creatinine by absorption and liberation from Lloyd's reagent. For further information on this substance the reader is referred to the original observations of Gaebler.

We have never found more than 0.5 mgm. per cent of non-creatinine chromogenic substance in the plasma of dogs fed upon our cracker meal diet or upon meat, and consequently when the plasma creatinine level is raised to 10 mgm. per cent or higher, these substances are not a serious source of error in the determination of the creatinine concentration by Folin (1919) method. The concentration of non-creatinine chromogenic substances in the relatively dilute urines which we have analyzed has not exceeded 1.0 mgm. per cent, which amount is, of course, so small that it also can be neglected in experiments in which large quantities of creatinine are administered. The excretion of non-creatinine chromogenic substances and the question of the origin of the creatinine present in normal urine, will be referred to in the latter part of this paper.

The secretion of exogenous creatinine. The first experiments which we wish to describe were made with dog 36; we have used this dog in many previous experiments and consequently have numerous data on her relative to the excretion of urea, xylose and sucrose under various conditions. The first three experiments (expts. 140, 141 and 143) concern the excretion of xylose and creatinine while the dog was on a maintenance diet of cracker meal, butter and sugar, and when the glomerular (xylose) clearance was at a minimum. In these experiments varying quantities of creatinine were administered subcutaneously, along with constant quantities of xylose by stomach. For the detailed data the reader is referred to table 1 and its accompanying protocols. The results of these experiments show clearly that the rate of creatinine excretion relative to the plasma level of creatinine—i.e., the creatinine clearance—is greater by about 40

¹ We have observed that methylcreatinine, benzylcreatinine and benzoylcreatinine (the latter prepared either from benzoylchloride or benzoic anhydride) are absorbed by Lloyd's reagent. It is obvious, therefore, that such substances would behave like creatinine in any method depending upon the use of this absorbent and probably also in any method based upon the conversion of creatinine to creatine. We are indebted to Dr. Isador Greenwald for the substituted creatinines used in making the above tests.

TABLE 1

A comparison of xylose and creatinine clearances at varying plasma levels of creatinine

PERIOD	TOTAL CON- CURRENT TIME	URINE FLOW PER MINUTE	XYLOSE		CREATININE		CM = $\frac{UV}{P}$ / S.A.		$\frac{\text{CM CREATININE}}{\text{CM XYLOSE}}$	$\frac{\text{CM UREA}}{\text{CM XYLOSE}}$
			Plasma	Urine	Plasma	Urine	Xylose	Creat- inine		

Expt. 140. Dog 36										
	minutes	cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.				
1	20	8.50	170.5	868	55.9	422	60.1	88.9	1.48	
2	40	5.20	173.5	1,225	52.7	527	51.0	72.2	1.42	
3	60	5.75	175.5	1,164	45.7	427	53.0	74.6	1.41	
4	81	5.47	176.5	1,234	43.0	406	53.1	71.7	1.35	
Average:									(1.41)	

Expt. 141. Dog. 36										
1	20	6.50	168.5	1,150	42.5	395	61.6	83.9	1.36	
2	42	5.10	174.4	1,477	39.2	473	60.0	85.5	1.42	
3	60	3.06	159.6	2,010	34.2	593	53.5	73.7	1.38	
4	85	0.60*	135.2	2,900	28.8	863	17.9*	25.0*	1.40	
Average:									(1.39)	

Expt. 143. Dog 36										
1	21	3.33	92.2	1,100	31.2	544	55.7	80.6	1.45	0.687
2	41	4.05	98.9	926	27.2	376	52.7	77.8	1.48	0.700
3	62	4.95	103.0	921	23.2	283	61.5	83.8	1.36	0.657
4	82	5.10	105.2	930	20.7	251	62.6	85.9	1.37	0.645
Average:									(1.42)	

Expt. 159. Dog 36										
In this experiment "true" creatinine values for plasma and urine are given										
1	31	3.74	125.0	1,604	25.10	406	66.7	84.1	1.26	
2	60	5.07	135.8	1,357	22.50	297	70.2	92.9	1.32	
3	92	5.72	134.0	1,299	18.60	244	77.0	104.2	1.35	
4	121	3.40	109.8	1,900	16.40	383	81.7	110.4	1.35	
5	150	1.62	83.4	2,750	14.50	670	74.2	103.9	1.40	
6	179	1.14	59.7	2,780	12.00	803	73.7	105.8	1.44	
(322)	** See note A: more xylose administered									
7	346	5.70	169.5	1,579	5.56	76	73.7	108.2	1.47	
8	376	3.77	175.5	2,350	5.14	90	70.1	91.7	1.35	
9	410	3.18	133.3	2,372	4.35	107	78.5	108.7		
10	436	1.62	98.1	3,150	3.85	173	72.3	101.2	1.40	
11	466	1.14	75.5	3,300	3.50	222	69.3	99.8	1.44	
12	496	0.87	52.6	3,040	3.00	253	69.7	101.9	1.46	
Average:									(1.39)	

* Urine spilled.

** Urine from washout period discarded.

to 50 per cent than is the rate of xylose excretion relative to the plasma level of xylose. (Our figures are corrected to the plasma clearance per square meter of body-surface area, as in previous papers, to facilitate the comparison of various dogs.)

On the basis of the evidence, presented by Jolliffe, Shannon and Smith (1932), that the rate of excretion of xylose relative to the plasma concentration measures within a few per cent the quantity of glomerular filtrate, or the glomerular clearance, the results of experiments 140, 141 and 143 must be accepted as evidence that a considerable quantity of creatinine is removed from the blood by some means other than, and in addition to that which is cleared by glomerular filtration. We infer that this additional quantity of creatinine represents a tubular excretion of this substance from the post-glomerular plasma, and we will therefore refer to it as the *tubular clearance*, in contradistinction to the *glomerular clearance*. Thus, in period I of experiment 140, the glomerular clearance of creatinine (theoretically the same as the glomerular clearance of xylose) is 60.1 cc. per square meter per minute, and the tubular clearance is 28.8 cc. per square meter per minute (88.9-60.1); we may conveniently identify the relative values of these by saying that the tubular clearance is 48 per cent of the glomerular clearance.

The difference between the xylose clearance and the creatinine clearance cannot be due to the excretion of what we may designate the normal or endogenous creatinine because the normal excretion of creatinine for a dog of this size is not greater than 0.3 mgm. per minute, whereas the average discrepancy between the xylose clearance and the creatinine clearance in experiment 140 is 22.3 cc. of plasma per minute containing 49.3 mgm. per cent or 11 mgm.,—i.e., 33 times the normal excretion. Instead, we are led to conclude that preformed creatinine is removed from post-glomerular blood by tubular activity and secreted directly or indirectly into the tubular urine.

The first question which arises in connection with the tubular secretion of creatinine is its relationship to the concentration of this substance in the plasma. In experiments 140, 141 and 143 the plasma level varied from 55.9 down to 20.7 mgm. per cent. There are irregularities in the relative tubular clearance from 37 to 48 per cent of the glomerular clearance, but the variations are distributed irregularly and are probably due to experimental error; if averages are taken for each experiment, as is indicated in table 1, the relative tubular clearance for the three experiments at average plasma levels of 49.3, 36.2 and 25.6 respectively, was 41, 39 and 42 per cent. In experiment 159, performed on this same dog two and a half months after experiment 143, the plasma level ranged from 25.0 down to 3.0 mgm. per cent; in this experiment the tubular clearance was 38 per cent of the glomerular clearance. (In expt. 159 the true creatinine, as

determined by the method described in the Appendix, is given for the plasma while the urine concentration in this experiment and the plasma and urine concentrations in expts. 140, 141, and 143 are for the total chromogenic substance.) These four experiments indicate then, that at plasma levels above 3.0 mgm. per cent the tubular excretion of creatinine is a linear function of the plasma concentration and that the tubular clearance is therefore independent of the plasma concentration. Since the glomerular excretion of creatinine must be assumed to be a linear function of the plasma concentration so long as the rate of glomerular filtration is constant, it follows that, all other conditions being equal, the total excretion of creatinine will likewise bear a linear relationship to the plasma concentration. MacKay and Cockrill (1930) have demonstrated that such a linear relationship exists in the rabbit under "standard conditions," but they have erroneously argued that this fact may be taken as evidence that creatinine is excreted in this animal solely by glomerular filtration. On the contrary, it is quite possible that the tubular excretion is proportional to the plasma concentration in the rabbit as in the dog, in which case the total excretion would bear the same relationship.

The influence of diet upon the tubular excretion of creatinine. It is held on good grounds that the blood to the renal tubules in the mammal is supplied entirely from the efferent glomerular arterioles. Except for such changes in glomerular filtration as might result from changes in local glomerular pressure it would be expected, therefore, that the glomerular clearance and the tubular clearance would be affected in the same direction by changes in number of active glomeruli and in blood flow through the kidney, and where the tubular excretion is a linear function of the plasma concentration, as in the case of creatinine, these should be affected to approximately the same extent.

It has been shown in a previous paper that the rate of glomerular filtration (and the urea clearance) can vary in a single dog as much as three and a half fold in relation to the dietary regime (Shannon, Jolliffe and Smith, 1932) and it is known that the creatinine clearance increases and decreases under these same conditions (Jolliffe and Smith, 1931). It therefore seemed important to examine the effects of diet on the tubular clearance of creatinine relative to the glomerular clearance as measured by xylose.

With this point in mind we transferred dog 36 from the cracker meal diet which had been used throughout the above experiments, and upon which the glomerular clearance was low (50 to 60), to a mixed diet. In the next two experiments (expts. 147 and 149, table 2) after seven days on the mixed diet, and after eating a meat meal, the glomerular clearance ranged from 90 to 97. Thus by dietary means the glomerular clearance was nearly doubled, but the proportion between the glomerular clearance and the tubular clearance of creatinine remained fairly constant, the latter

TABLE 2

Effect of diet and erythroltetranitrate on xylose and creatinine clearances

PERIOD	TOTAL CON- CURRENT TIME	URINE FLOW PER MINUTE	XYLOSE		CREATININE		CM = $\frac{UV}{P}$ / S.A.		CM CREATININE CM XYLOSE	CM UREA CM XYLOSE
			Plasma	Urine	Plasma	Urine	Xylose	Creat- inine		
Expt. 147. Dog 36										
	minutes	cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.				
1	30	1.90	95	3,255	30.9	1,404	90.4	119.7	1.32	0.790
2	60	1.70	89	3,645	27.4	1,446	96.7	124.8	1.29	0.763
3	87	1.59	79	3,470	24.7	1,417	97.0	126.5	1.30	0.796
Average:									(1.30)	
Expt. 149. Dog 36										
1	31	2.45	146	3,895	35.6	1,290	90.8	123.0	1.36	0.807
2	61	2.20	130	4,065	28.8	1,244	95.5	131.8	1.38	0.777
3	91	1.66	100	4,085	24.9	1,333	94.2	123.5	1.31	0.764
Average:									(1.35)	
Expt. 158. Dog 36										
1	22	2.68	121	3,285	27.4	985	101.0	133.8	1.33	
2	44	2.77	127	3,390	27.6	990	102.7	138.0	1.34	
3	65	3.23	134	3,020	27.0	790	101.2	131.3	1.30	
See note A: E. T. N. administered							Average:			
4	95	4.23	136	2,000	28.4	543	86.4	112.5	1.30	
5	117	2.27	134	3,450	26.5	896	81.2	106.8	1.31	
6	151	1.82	102	3,470	22.5	995	86.1	111.7	1.30	
7	181	1.53	74	3,110	18.5	1,015	89.3	116.5	1.30	
8	215	1.09	56	3,300	14.7	1,143	89.2	117.7	1.31	
Average:									(1.31)	
Expt. 157. Dog 43										
1	15	2.27	199	3,290	82.0	1,547	40.8	46.6	1.14	
2	35	2.40	206	3,140	75.5	1,345	39.7	46.5	1.16	
3	56	2.38	209	3,305	70.2	1,245	40.9	45.9	1.12	
(99) ** See note A: E. T. N. administered.							Average:			
4	127	3.46	246	3,740	60.0	1,111	57.2	69.6	1.21	
5	164	3.24	224	3,830	55.0	1,053	60.2	67.4	1.10	
6	201	2.62	200	4,070	51.0	1,178	58.0	65.8	1.13	
7	235	2.21	167	3,970	47.0	1,333	57.1	68.1	1.19	
8	265	1.94	133	4,630	41.9	1,646	73.4	82.8	1.12	
9	295	1.53	104	3,540	38.7	1,507	56.6	64.8	1.14	
Average:									(1.15)	

** Urine from washout period discarded.

dropping only from an average of 40 per cent of the glomerular clearance on the cracker meal diet (expts. 140, 141, 143 and 159) to 33 per cent in the mixed-diet and post-prandial experiments (expts. 147 and 149). This result suggests that only a small part of the increased glomerular activity in the latter experiments is due to increased glomerular pressure, and that the greater part of the increase in glomerular activity is accompanied by an increase in blood flow to the tubules.

To examine this point farther, in the last experiment and while the dog was still on a mixed and meat diet, a vasodilator was administered after three control periods (expt. 158). The vasodilator was erythroltetranitrate which was selected because it has a slow and prolonged action. During the three control periods the glomerular clearance was 100; this dropped to 80.9 in the second period after the erythroltetranitrate was injected. Throughout the course of the experiment, however, the relative tubular clearance of creatinine remained remarkably constant at 30 to 33 per cent of the glomerular clearance.

In another experiment of this same type (expt. 157), but performed on another dog which was being fed the cracker meal diet, and which therefore had a low glomerular clearance, the erythroltetranitrate had a reverse effect; the glomerular clearance rose from 40 before the injection of the drug to a maximum of 73 afterward. It is of course impossible to interpret these results accurately in terms of immediate causes, since the drug probably produces opposing effects; while it would probably tend to increase the number of open or active glomeruli and hence the total filtering surface and blood flow, it would at the same time be expected to decrease the rate of glomerular filtration by lowering the local glomerular and the systemic arterial pressure. Whether the net rate of glomerular filtration would increase or decrease would then depend on the predominance of these opposing effects. But the relative constancy of the ratio of the glomerular to the creatinine clearance in the above experiments in which the glomerular clearance was nearly doubled by diet, and profoundly modified in diverse directions by a vasodilator drug, indicates that changes in the rate of glomerular filtration are for the most part accompanied by parallel changes in blood flow to the tubules.

(It may be noted that dog 43, used in this last experiment, showed a relatively lower creatinine clearance than did dog 36; under comparable conditions the tubular clearance of creatinine was 40 per cent of the glomerular clearance in dog 36, 15 to 30 per cent in dog 43 and 17 to 31 per cent in dog 30.)

*The effect of phlorizin on the secretion of exogenous creatinine.*² Clark

* Poulsson (1930) has compared the excretion of creatinine with glucose in phlorizinized dogs. With one exception, the U/P ratios for creatinine in his experiments were greater than for glucose, the glucose/creatinine ratios varying from 1.10

TABLE 3
Effect of phlorizin on secretion of creatininc, etc.

PERIOD	TOTAL CONCUR- RENT TIME	URINE FLOW PER MINUTE	XYLOSE		CREATININE		GLUCOSE		CM = $\frac{UV}{P}$ / S.A.			CM CREATININE CM XYLOSE	CM GLUCOSE CM XYLOSE	CM CI CM XYLOSE
			Plasma	Urine	Plasma	Urine	Plasma	Urine	Xylose	Creati- nine	Glucose			
Expt. 155. Dog 30														
1	16	2.00	151	3,595	87.8	2,438			55.3	64.6		1.17		0.051
2	35	3.10	161	3,540	89.2	2,397			53.7	65.6		1.22		0.037
3	63	2.00	165	3,880	89.1	2,420			54.7	63.2		1.16		0.019
See note A: phlorizin administered									Average: (1.19)					
4	79	2.43	163	2,765	83.5	1,471			48.0	49.8		1.04		0.171
5	100	3.28	157	2,025	79.0	1,053			49.3	50.8		1.03		0.180
6	130	2.06	130	2,590	75.0	1,505	102	1,957	47.7	48.1	46.0	1.01	0.962	0.042
See note B: more phlorizin administered									Average: (1.02)					
7	150	1.90	116	2,313	70.0	1,385	102	1,988	44.1	43.7	43.1	0.99	0.978	0.081
8	170	2.05	104	2,010	65.5	1,238	103	1,860	46.1	45.1	43.1	0.98	0.935	0.086
9	190	1.95	100	2,090	63.5	1,415	104	2,312	47.4	50.5	50.4	1.06	1.06	0.078
									Average: (1.00)			(0.984)		
Expt. 160. Dog 36														
														CM UREA CM XYLOSE
1	20	3.50	186	2,272	24.4	412	77	0.0	59.4	82.0	0.0	1.38	0.0	0.752
2	49	7.62	192	3,000	22.0	457	78	0.0	56.8	75.5	0.0	1.33	0.0	0.756
3	76	2.73	177	2,886	19.5	390	76	0.0	61.8	75.8	0.0	1.23	0.0	0.726
See Note A: phlorizin administered									Average: (1.32)			(0.0)		0.743
4	106	2.63	145	2,150	17.5	273		1,158	54.2	56.9		1.05		0.736
5	135	2.34	115	2,069	15.6	278	89	1,574	58.4	57.9	59.4	0.99	1.02	0.672
6	162	2.14	87	1,800	13.9	288	75.6	1,535	61.6	61.6	60.3	1.00	0.98	0.673
									Average: (1.01)			(1.00)		(0.693)

and Smith (1932) have found that, as judged by the simultaneous excretion of xylose, creatinine is abundantly secreted by the elasmobranch kid-

to 1.27 in one experiment, from 1.05 to 1.31 in a second, and from 0.91 to 1.20 in a third. Nevertheless, he concluded that the correspondence between these ratios was such as to establish that creatinine is excreted exclusively by filtration.

Apart from our present demonstration that phlorizin abolishes the tubular excretion of creatinine, we believe that Poulsson's experiments are open to several criticisms; the plasma and urine sugars were determined by different analytical methods and the plasma sugar was determined by the Hagedorn-Jensen method in which phlorizin has

ney. This secretory activity was observed to be depressed by phlorizin, and in some instances it was completely abolished so that the total excretion of creatinine decreased until it corresponded to the quantity excreted

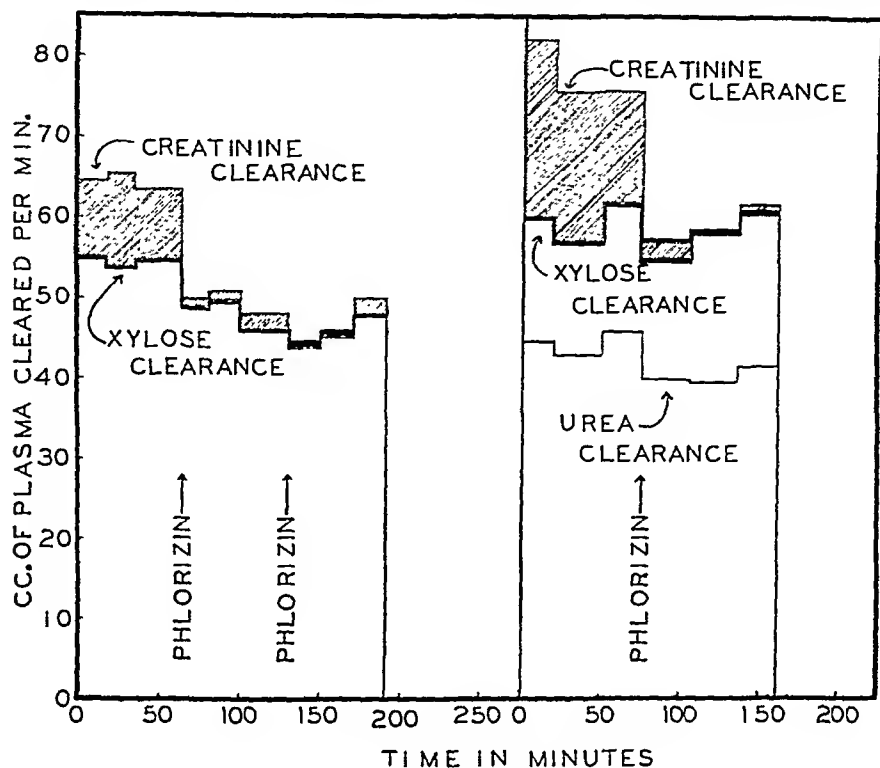


Fig. 1. The effect of phlorizin on the tubular secretion of creatinine. The creatinine clearance exceeds the xylose (glomerular) clearance in the normal dog by 15 to 50 per cent; when phlorizin is administered the tubular secretion of creatinine is arrested and consequently the creatinine clearance drops to the xylose or glomerular level. Phlorizin does not significantly affect the xylose or urea clearances, but it brings the glucose clearance up to the xylose clearance.

by glomerular filtration (this being the minimum, of course, if we assume that no creatinine is reabsorbed by the tubules).

a strong reducing action (cf. Marshall, 1932); the urine sugars were determined by Benedict's titration method, in which phlorizin interferes with the end point. Since something over 200 mgm. per kgm. of phlorizin were given subcutaneously we may expect, if this substance is absorbed as quickly as sucrose, for example, to obtain from 10 to 30 mgm. per cent of phlorizin in the plasma and 20 to 50 times this concentration in the urine. In one experiment the non-glucose reducing substance in blood was determined by prolonged fermentation with yeast, but since this was not done on each plasma sample this precaution would not circumvent the difficulty. In addition a relatively enormous correction for the reducing power of creatinine, amounting to 50 per cent as its glucose equivalence, was necessary with the Hagedorn-Jensen method. These sources of error raise a serious doubt about the accuracy of the glucose U/P ratios observed by Poulsson.

It is of particular interest, therefore, to observe the effects of phlorizin on the excretion of creatinine in the dog. In experiments 155 and 160 (table 3) the normal xylose and creatinine clearances were observed in several control periods; phlorizin was then administered by intravenous and subcutaneous injection. (Our phlorizin is carefully purified by recrystallization from alcohol, as recommended by Lusk, 1928.) It will be seen from these experiments, which are also illustrated in figure 1, that phlorizin instantly and completely abolishes the extra excretion of creatinine, reducing the total creatinine clearance to the glomerular level. No explanation can be advanced for this action of phlorizin, but it may be reiterated, as Clark and Smith (1932) have pointed out, that a phlorizinized animal is hardly suitable for the examination of "normal" renal function.³

The action of phlorizin in arresting the tubular secretion of creatinine (as we interpret the excessive creatinine clearance observed in the normal animal) without significantly affecting the xylose or urea clearances is a further though indirect validation of our thesis that xylose measures the rate of glomerular filtration.⁴

³ In view of the action of phlorizin in arresting the secretion of creatinine we have examined its effects on the excretion of a few other substances. In experiment 155 phlorizin did not significantly affect the excretion of Cl; the Cl clearance varied slightly immediately after the phlorizin was given, but it did not in any instance approach the glomerular clearance as would have been the case had this drug depressed the reabsorption of Cl to any considerable extent. In experiment 160 no creatine and only the faintest traces of phosphate (less than 0.1 mM per liter) were found in the urine either before or after phlorizin, so that, if these substances are filtered from the plasma, this drug does not arrest their reabsorption by the tubules. The excretion of water is obviously not affected by phlorizin except as the glucose constitutes an added osmotic factor in the urine (cf. expts. 155 and 160 in this paper, and expts. 87, 88, 92, 96 and 100 reported by Jolliffe, Shannon and Smith, 1932). Therefore the blocking of the reabsorption of glucose and of the secretion of creatinine remain the only two renal effects known to be exerted by this drug.

⁴ Attention should be called again to the significance of the fact that phlorizin does not affect the excretion of xylose relative to urea (see the ratios of the xylose:urea clearances in expt. 160) as was pointed out by Jolliffe, Shannon and Smith (1932). This fact is substantial evidence that the effect of phlorizin consists of depressing the excretion of creatinine rather than increasing the excretion of xylose, since it is very unlikely that the drug would block a hypothetical reabsorption of urea and xylose to exactly the same extent. Moreover, the usual effect of phlorizin on the xylose clearance is not to increase it, as one would have to assume if the excretion of creatinine were taken as a measure of glomerular filtration, but rather to decrease the clearance as though there were some irritant action exerted upon the glomeruli. It is interesting to note in this connection that Marshall and Grafflin (1932) have found that relatively small doses of phlorizin cause the glomeruli in the sculpin to close up completely for several hours. No such marked result has been observed in the dog, although the administration of phlorizin is usually followed by a decrease in the xylose clearance. This effect may be due in part to hemolysis, since we have always given part of our phlorizin intravenously and since hemoglobin is known to produce a transient decrease in renal blood flow.

The excretion of endogenous creatinine. It is impossible by available methods to determine accurately the normal true creatinine in the plasma, and more particularly in urine, in animals to which xylose has been administered because of the interference of the sugar, as pointed out in the description of our creatinine method. Consequently it has not been possible for us to compare the excretion of xylose with the excretion of normal or endogenous creatinine. We have, however, made analyses on the plasma and urine of dogs which never had received injections of creatinine with the idea of determining whether or not the normal rate of excretion of creatinine falls within the same range as in dogs to which creatinine had been administered.

TABLE 4

The distribution of creatinine and non-creatinine chromogenic substance in normal plasma and urine

DOG	SURFACE AREA	SPECIMEN	(1)	(2)	(3)	(4)	U/P	V	(5)	DIET
			TOTAL CHROMOGENIC SUBSTANCE	AFTER LLOYD'S	CREATININE BY DIFFERENCE	CREATININE BY RECOVERY			UV/SA. P	
	sq. m.		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.		cc. per mm.		
49	0.70	Plasma	0.84	0.17	0.67	0.67	32.6	1.02	47.5	Cracker meal
		Urine	22.70	0.83	21.87	21.50				
59	0.42	Plasma	0.77	0.15	0.62	0.65	21.7	1.19	61.6	Mixed
		Urine	14.00	0.52	13.48	12.90				
60	0.44	Plasma	0.53	0.17	0.36	0.52	57.2	0.64	83.3	Mixed
		Urine	21.30	0.68	20.62	25.00				

Data bearing on this point are given in table 4. In these analyses we have determined the total chromogenic substance in plasma and urine, using an aliquot of 2 cc. of the former and 1 cc. of the latter (column 1); the non-creatinine chromogenic substance remaining after extraction with Lloyd's reagent (column 2); true creatinine, as calculated by difference between (1) and (2); creatinine recovered from the Lloyd's reagent used in (2) by treatment with MgO (column 4).

Confirming Behre and Benedict (1922), Gaebler and Keltch (1928) and Gaebler (1930), we find a considerable moiety of the total chromogenic substance in plasma is not absorbed on Lloyd's and conclude that it is not creatinine. In other experiments we have found larger quantities of non-creatinine chromogenic substance than are given in table 4; for example, in dog 36, experiment 159, this moiety was 0.5 ± 0.05 mgm. per cent in all

plasma samples, and in another dog we observed 0.61 mgm. per cent. It is apparent that there may be sufficient non-creatinine chromogenic substance in plasma to introduce a serious error in creatinine clearance determinations based upon total plasma creatinine unless the plasma creatinine is 10 mgm. per cent or higher. It is interesting to observe that the non-creatinine chromogenic substance is not concentrated in the urine to the same extent as is creatinine (or even, we may suppose, as xylose); if this moiety is filtered through the glomeruli, it must be reabsorbed by the tubules.

Regarding the recovery of the absorbed creatinine from the Lloyd's reagent, it will be noted that in two instances (dogs 49 and 59) this recovery was almost perfect. In the third instance (dog 60) more creatinine was recovered from both the plasma and the urine than had been absorbed; it may be that this excessive recovery signifies the presence of the substance which Gaebler has described, and which is converted to creatinine by treatment with Lloyd's.

When the creatinine clearance is calculated from the true creatinine in the plasma and urine (column 3) of the above experiments, values are obtained (see column 5, table 4) which lie well within the range of the creatinine clearances which have been reported previously by Jolliffe and Smith (1931) and in this paper (i.e., between 40 and 150 per sq. m.). Behre and Benedict (1922) suggested, when they ascertained that not all the chromogenic substance in the blood is creatinine, that there might not be enough creatinine in the blood to account for the normal excretion of this substance. The above observations indicate, on the contrary, that it may not be necessary to infer the renal formation of creatinine from some precursor in order to account for the normal excretion of this substance. We make this point tentatively, however, because the experimental method is none too good, and at best it is quite non-specific since it includes substituted creatinines (methyl-creatinine, etc.) in the moiety which we have called creatinine.

SUMMARY

In normal dogs to which creatinine has been administered the creatinine clearance exceeds the xylose (glomerular) clearance by 15 to 40 per cent. This excessive excretion of creatinine is interpreted as due to the tubular secretion of this substance from the post-glomerular blood.

The tubular secretion of creatinine appears to be a linear function of the plasma concentration below 50 mgm. per cent, and therefore the tubular clearance is independent of the plasma concentration. Since the glomerular clearance is theoretically independent of the plasma concentration, it follows that the total creatinine clearance is likewise independent of the plasma concentration.

Changes in the glomerular clearance effected by diet and by erythroltetranitrate are accompanied by nearly proportional changes in the same direction in the creatinine clearance, indicating that the former are accompanied by nearly proportional changes in blood flow to the tubules.

Phlorizin completely arrests the tubular secretion of exogenous creatinine so that the total creatinine clearance is reduced to the level of the xylose (or glucose) clearance.

The excretion of endogenous creatinine is discussed briefly in the text.

Protocols accompanying tables 1, 2, and 3. (With the experiments on dog 36 arranged in sequence)

Experiment 140. Dog 36. April 8th. Weight 16.2 kgm., S. A. 0.72 sq. m. Cracker meal diet since March 16th. Twenty-four grams xylose in 640 cc. water by stomach at 9:10 a.m. Eight grams xylose in 320 cc. water by stomach and 8 grams creatinine in 100 cc. water subcutaneously at 9:45 a.m. Period 1 began at 10:42 a.m. Blood drawn at middle of each urine collection period.

Experiment 141. Dog 36. April 11th. Procedure same as in experiment 140 except that 6.4 grams creatinine were given.

Experiment 143. Dog 36. April 15th. Procedure same as in experiment 140 except that 4.0 grams creatinine were given.

Experiment 147. Dog 36. April 25th. Fed 4 lbs. meat April 18. Ate very little meat thereafter. Ate mixed diet April 22nd. Would not eat on April 23rd and 24th because of hot weather. On April 25th ate 850 grams raw beef at 7:20 a.m. Twenty-four grams xylose in 640 cc. water by stomach at 9:30 a.m. Eight grams xylose in 160 cc. water by stomach and 8 grams creatinine in 100 cc. water subcutaneously at 10:00 a.m. Period 1 began at 11:11 a.m. Blood drawn at middle of urine periods.

Experiment 149. Continuation of experiment 147. Thirty-two grams xylose in 200 cc. water by stomach at 6:35 p.m. and 8 grams creatinine in 100 cc. water subcutaneously at 7:10 p.m. At 7:20 ate about 250 grams cooked liver. Period 1 began at 8:20 p.m. Bloods as before.

Experiment 158. Dog 36. May 30th. Mixed diet since April 25th. Meat offered May 26th-29th but did not eat well because of hot weather. Twenty-four grams xylose in 320 cc. water by stomach at 8:45 a.m. Sixteen grams xylose in 640 cc. water by stomach and 8 grams creatinine in 100 cc. water subcutaneously at 10:10 a.m. Period 1 began 11:12 a.m. Note A: at end of period 3 one grain of erythroltetranitrate subcutaneously and two grains by stomach with 150 cc. water. Bloods 5 and 6 interpolated. Hematocrit 31.5 per cent. Dog obviously affected by erythroltetranitrate; weak at end of experiment.

Experiment 159. Dog 36. June 3rd. Cracker meal diet since May 30th. Five hundred cubic centimeters water had been given by stomach every day. Twenty-four grams xylose in 640 cc. water by stomach at 8:00 a.m. Eight grams xylose in 320 cc. water by stomach and 4.8 grams creatinine in 75 cc. water subcutaneously at 9:30 a.m. Period 1 began at 9:30 a.m. Note A: 16 grams xylose in 640 cc. water by stomach at 12:30 a.m. Twelve grams xylose in 320 cc. water by stomach at 1:00 p.m. Bloods drawn near middle of each urine period and interpolated to exact middle. True plasma creatinine determined as described in appendix. Non-creatinine chromogenic substance in plasma averaged 0.5 mgm. per cent, expressed as creatinine.

Experiment 160. Dog 36. June 7th. Cracker meal diet since May 30th. Twenty-four grams xylose in 640 cc. water by stomach at 9:00 a.m. Eight grams xylose in 320 cc. water by stomach and 8 grams creatinine in 100 cc. water subcutane-

ously at 9:30 a.m. Period 1 began at 10:34. Note A: at end of period 3, 3.2 grams phlorizin in NaHCO_3 solution intravenously and same quantity subcutaneously. Bloods drawn near middle of each urine period and interpolated to exact middle.

Experiment 157. Dog 43. May 27th. Weight 20 kgm., S. A. 0.92 sq. m. Cracker meal diet since May 6th. Thirty grams xylose in 800 cc. water by stomach at 8:30 a.m. Twenty grams xylose in 400 cc. water by stomach and 10 grams creatinine in 120 cc. water subcutaneously at 9:00 a.m. Period 1 began at 10:21 a.m. Note A: at end of period 3 one grain erythroltetranitrate subcutaneously and one and one-half grains, together with 10 grams xylose and 5 grams creatinine in 200 cc. water by stomach. Bloods drawn at middle of each urine period.

Experiment 155. Dog 30. May 23rd. Weight 20 kgm., S. A. 0.86 sq. m. Cracker meal diet since May 6th. Thirty grams xylose in 800 cc. water at 8:15 a.m. Thirty grams xylose in 400 cc. water by stomach and 20 grams creatinine in 200 cc. water subcutaneously at 9:00 a.m. Period 1 started at 10:00 a.m. Note A: at end of period 3, 4 grams phlorizin in NaHCO_3 solution intravenously and same quantity subcutaneously. Note B: at end of period 6, 4 grams phlorizin in NaHCO_3 solution intravenously. Bloods drawn at middle of urine periods. Dog vomited in period 4 and period 7.

APPENDIX. The method which we have used for the determination of true creatinine is that of Folin (1919) with a simple modification of the procedure used by Gaebler (1930) in which creatinine is absorbed in Lloyd's reagent. Our method is described in full below:

Total chromogenic substance. Ten cubic centimeters of a 1:10 tungstate filtrate of plasma (prepared by adding 7 volumes of water, one volume of 5 per cent sodium tungstate and one volume of 0.33 N H_2SO_4 to one volume of plasma) or a quantity of diluted urine containing from 0.2 to 0.8 mgm. of creatinine, are placed in 20 × 200 mm. test tubes and made up to a total volume of 10 cc. Standard creatinine solutions are prepared in the same manner. Five cubic centimeters of alkaline picrate, freshly prepared by adding 1 volume of 10 per cent NaOH to 5 volumes of freshly saturated, filtered picric acid solution are added to each tube and thoroughly mixed. Colorimetric comparison is made after 12 minutes. The picric acid used is recrystallized by Benedict's (1929) method, and the saturated solution prepared by mechanical shaking just before use.

Non-absorbable chromogenic substance. Ten cubic centimeters of a 1:5 tungstate filtrate of plasma, or a quantity of urine containing not over 5 mgm. of creatinine are placed in a 15 cc. centrifuge tube with sufficient water to bring the total volume to 12 cc. One drop of concentrated HCl and about 300 mgm. of Lloyd's reagent (measured roughly from a small spoon) are added to each tube; the tubes are stoppered with close fitting stoppers and shaken for 10 minutes. (Standard creatinine solutions are treated in the same manner for subsequent use, as described below.) The Lloyd's reagent absorbs the creatinine and leaves only the non-creatinine chromogenic substance in the solution. The tubes are centrifuged until the sediment is well packed, the supernatant fluid is then decanted into convenient receptacles and the empty tubes are left inverted to drain for subsequent use. For the determination of the non-absorbable chromogenic substance 10 cc. of the decanted fluid are placed in a 25 or 50 cc. conical centrifuge tube. Standards containing 0.001, 0.002, 0.003, etc., mgm. of creatinine are prepared in 10 cc. of water and placed in similar centrifuge tubes to facilitate preliminary comparison. Five cubic centimeters of alkaline picrate are added, and during the development of color the unknowns are centrifuged to throw down the slight precipitate which is produced by the alkali. Colorimetric comparison is made as usual after 12 minutes. A transient greenish color develops in the plasma filtrates so treated, but this disappears after 3 to 5 minutes and does

not interfere with the subsequent matching. The quantity of apparent chromogenic substances as determined colorimetrically must be multiplied by 12/10 to allow for the 10 cc. aliquot removed from the 12 cc. of extracted fluid. We find that 300 mgm. of Lloyd's reagent will completely remove 15 mgm. of creatinine. (After extraction of creatinine (our sample) there remains a slight residuum of chromogenic substance amounting to less than 0.5 per cent of the original creatinine; since our sample of Lloyd's reagent does not give any perceptible chromogenic blank by the above method, we interpret this residuum to be a non-absorbable chromogenic impurity in the creatinine.)

This method has been tested on a sample of urine which was found by a preliminary analysis to have 0.0023 mgm. per cubic centimeter of non-absorbable chromogenic substance; the recovery from 2, 4, 6 and 10 cc. of this urine was 0.0047, 0.0083, 0.0141 and 0.0240 mgm., showing a good proportionality between the color development and the quality of urine taken for analysis.

Recovery of creatinine from the Lloyd's reagent. The creatinine absorbed on the Lloyd's reagent in the above analysis is recovered by the addition of 12 cc. of water and 100 mgm. of MgO to the sediment in each tube. The sediment is loosened with a stirring rod, and the tubes stoppered and shaken for 10 minutes. There is no need to wash the sediment before liberating the creatinine because the included water is small in amount and contains only the now relatively dilute non-absorbable chromogenic substance. The suspensions are then centrifuged, decanted, and creatinine determined as above on 10 cc. aliquots. Known quantities of creatinine, as suggested above, should be included in the series and treated in an identical manner in order to evaluate the dilution resulting from the water included in the sediment. We recover about 75 per cent of the absorbed creatinine, as contrasted to the theoretical 83.3 per cent expected from the 10/12 aliquot used. The lower recovery is due, of course, to dilution by the water included in the Lloyd's after centrifuging.

Gaebler (1930) recovered from Lloyd's reagent which had been shaken as above with tungstate and picrate filtrates of dog's plasma more creatinine than had been absorbed, as calculated from the difference in the filtrates before and after extraction, and he concluded that the blood contains a non-chromogenic substance (other than creatine) which is converted to creatinine by the treatment with the Lloyd's reagent. We are not primarily interested in this substance here because, so far as glomerular filtration is concerned, only the preformed creatinine in the plasma is significant. The preformed creatinine is given, so far as it can be determined at the present time, by the difference in total chromogenic substance before and after extraction with Lloyd's reagent. The method which we describe above cannot be considered to be more than tentative because, as Gaebler points out, it is not known whether the color produced in untreated filtrates containing both true creatinine and other chromogenic substances is additive. But our immediate problem required a more exact analysis of the creatinine-like substances in plasma than is possible with the Jaffe reaction alone, and we believe that as a first approximation this method serves our purpose.

Interference of xylose in creatinine determination. When 3 mgm. of xylose in 5 cc. of water are mixed with 5 cc. of alkaline picrate no color develops within 30 minutes; 6 mgm. of xylose give a color perceptibly darker than a blank within this time, and 15 mgm. of xylose give a marked color within 15 minutes.

When 0.05 mgm. of creatinine and 2.5 mgm. of xylose in 5 cc. of water are mixed with 5 cc. of alkaline picrate about 4.0 per cent enhancement of color is observed over a control without xylose at the end of 30 minutes. These proportions (1 mgm. of creatinine to 50 mgm. of xylose) therefore represent the maximal which can be used

with safety if readings are to be made from 12 to 20 minutes after the picrate is added. The highest proportion in any of our experiments is 1:34 (plasma, period 8, expt. 159) and the proportion is usually much less than this.

Because the creatinine:xylose ratio exceeds the above, it is impossible to determine either the creatinine or the non-creatinine chromogenic substance normally present in the plasma and urine when these contain large quantities of xylose, and it is for this reason that we have made observations on endogenous creatinine excretion only in dogs which had not received xylose.

Sugar method. In extension of the sugar methods described by Jolliffe, Shannon and Smith (1932) emphasis may be placed upon a few points: the individual tubes of yeast used for glucose absorption must be centrifuged in an identical manner, and we find that increased accuracy in the U/P ratio can be obtained by handling the corresponding plasma and urine tubes together in each step; i.e., in the preliminary packing, in the absorption, in the final centrifuging and in the water bath. We believe that the Folin sugar method is unsafe when more than 0.4 mgm. or less than 0.15 mgm. of glucose (or the glucose equivalent of xylose) is used. The pipettes used for measuring standards and unknowns should be cleaned in cleaning fluid after each use to insure uniform drainage.

We find that creatinine, added to plasma which is subsequently precipitated with Somogyi's copper method and cleared of glucose by yeast, shows a reduction of 10 per cent in the Folin sugar method (i.e., 10 mgm. creatinine = 1.0 mgm. glucose). The error in the xylose determination due to the reducing power of creatinine is compensated in the U/P ratio, however, when the urine is so diluted that the quantities of xylose in plasma and urine are about the same. Since we try to dilute the urine so that it can be read against the same and identical standard as the plasma, the error is reduced in nearly all of our figures to 1.0 per cent or less and no correction has been applied for it. The xylose values in our tables are given as read against a glucose standard, no correction being made for the diffusion of xylose into the yeast or for its specific reducing power. The diffusion of xylose into the yeast is considered in calculating the glucose content, however.

Note: Our attention has been called by the authors to a paper by McCance and Madders (1930) on the absorption of sugars from the human intestine, which we had unfortunately overlooked. McCance and Madders suggested that the excretion of non-metabolized sugars might be used to measure the rate of glomerular filtration, and by assuming that the sugar which is retained in the body after injection is uniformly distributed throughout the body-fluids (presumed to be 60 per cent by weight) in order to ascertain the concentration in these fluids, they calculated from the rate of sugar excretion that the glomerular filtrate must amount to 100 to 178 cc. per minute. Plasma concentrations were not determined, and no comment was made on the possible secretion or reabsorption of the sugar, but it was noted that the figures so obtained were in good agreement with those obtained by Rehberg (1926) from the rate of excretion of creatinine.

We regret that we were unaware of McCance and Madders' paper when we prepared our own on the excretion of non-metabolized sugars.

BIBLIOGRAPHY

- BENEDICT, S. A. 1928. Journ. Biol. Chem., lxxxii, 1.
BEHRE, J. A. AND S. A. BENEDICT. 1922. Journ. Biol. Chem., lii, 11.
CLARKE, R. W. AND H. W. SMITH. 1932. Journ. Comp. and Cell. Physiol., i, 131.
FOLIN, O. AND H. WU. 1919. Journ. Biol. Chem., xxxviii, 81.
GAEBLER, O. H. 1930. Journ. Biol. Chem., lxxxix, 451.

- GAEBLER, O. H. AND A. K. KELTCH. Journ. Biol. Chem., lxxvi, 337.
- JOLLIFFE, N. AND H. W. SMITH. 1931. This Journal, xcix, 101.
- JOLLIFFE, N., SHANNON, J. A., AND H. W. SMITH. 1932. This Journal, c, 301.
- LUSK, G. 1928. Science of nutrition. Philadelphia.
- MCCANCE, R. A. AND K. MADDERS. 1930. Biochem. Journ., xxiv, 795.
- MACKAY, E. M. AND J. R. COCKRILL. 1930. This Journal, xciv, 220.
- MARSHALL, E. K. 1930. This Journal, xciv, 1.
1932. Proc. Soc. Exptl. Biol. and Med., xxix, 971.
- MARSHALL, E. K. AND A. L. GRAFFLIN. 1928. Bull. Johns Hopkins Hosp., xliii, 205.
1932. Journ. Cell and Comp. Physiol., i, 161.
- POULSSON, L. T. 1930. Journ. Physiol., lxix, 411.
- REHBERG, P. B. 1926. Biochem. Journ., xx, 447.

SOME MECHANISMS INVOLVED IN THE REGULATION OF THE CIRCULATION

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By means of the injection method (1, 2, 3, 4, 5, 6) one is able, in a single experiment, to determine the cardiac output (F), the stroke volume (SV), the mean (MCT), total and lesser circulation times, the volume of actively circulating blood in the heart, lungs and great vessels (V), and the total blood volume of man or of the dog.

The purpose of this investigation is to make determinations of these quantities in dogs, under the influence of cardio-vascular drugs, and under the influence of the arteriovenous fistula. There is no desire to analyze the response of the animal to therapeutic doses of the drugs involved, but rather to bring forward such considerations as may increase our understanding of the mechanism by which the circulation is controlled.

The experiments were performed as described in the above references. One hundred milligrams of "Brilliant Vital Red" were injected into the jugular vein. Consecutive samples were taken from the exposed femoral artery by means of a needle so bent that the sharp end was parallel to the artery and the other end pointed downward into the sampling tubes on the edge of a kymograph drum.

It was found that in cases where the serum was contaminated with hemoglobin, other pigments or with lipoids, that the colorimetric procedures could be carried out best when the small sample of plasma was diluted in 1 cc. of alcohol. When the alcoholic samples were stoppered and centrifugalized the precipitated proteins carried down all adventitious color and the dye in the alcoholic solution was proportional to the concentration of the dye in the plasma or serum. The lipoids made a clear solution in the alcohol and hence did not hinder colorimetry.

The results of these experiments are expressed in terms of surface area of the animal, as calculated from the formulae of Cowgill and Drabkin (7). Due to the fact that it was impossible for us to work with trained, unanesthetized dogs, it was thought that a dose of morphine, about one milli-

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gram per kilogram, would give conditions sufficiently near the basal (8, 9) to use as a starting point. The dogs were in a post-nutritive state.

Figure 1 is a typical time concentration curve of consecutive samples taken from the femoral artery of a dog under morphine alone, when 100

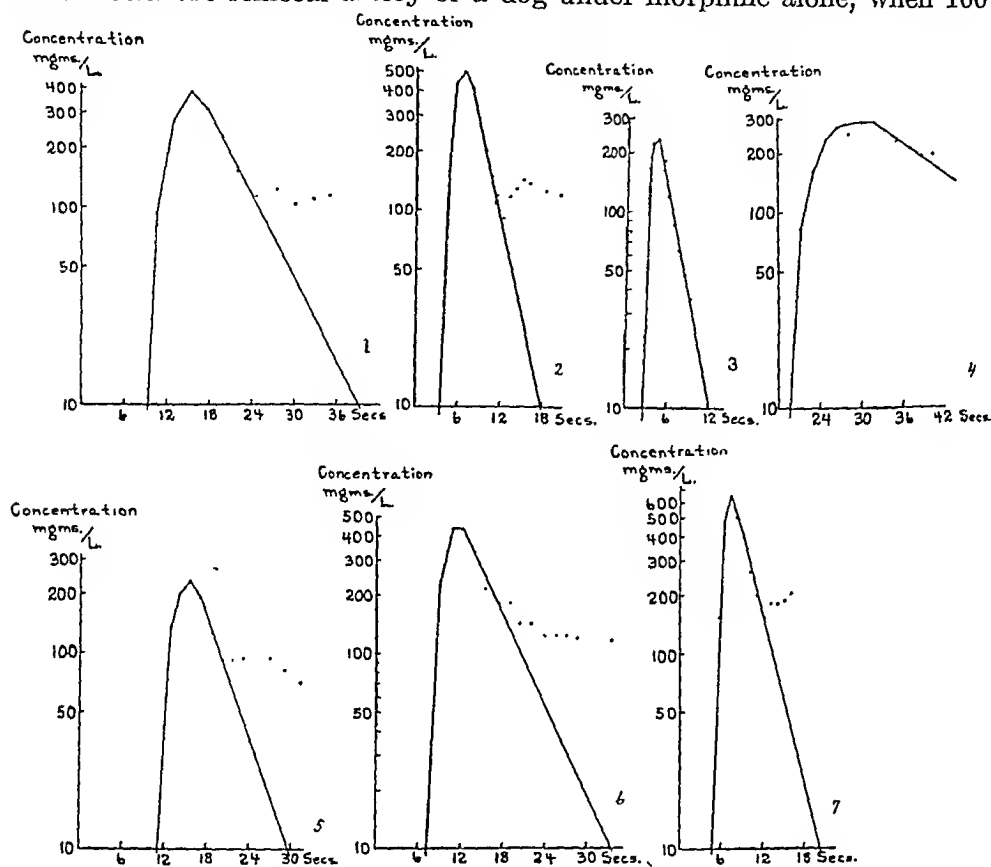


Fig. 1. Time concentration curve of dye in arterial blood after injecting 100 mgm. of Brilliant Vital Red into jugular vein. Dog under morphine; table 1, dog 2.

Fig. 2. Same as figure 1. Morphine; fast circulation; table 2, dog 6.

Fig. 3. Same as figure 1. Dog under morphine and atropine; table 3, dog 3.

Fig. 4. Same as figure 1. Morphine; 0.5 mgm. adrenin given intravenously 20 seconds before dye was injected. Table 4, dog 6.

Fig. 5. Same as figure 4. Adrenin given 60 seconds before dye. Table 5, dog 6.

Fig. 6. Same as figure 5. Morphine and atropine. Adrenin 20 seconds before dye. Table 6, dog 8.

Fig. 7. Same as figure 1. Inhalation of amyl nitrite begun 20 seconds before dye was injected. Table 8 dog 6.

mgm. of Brilliant Vital Red had been injected quickly into the jugular vein. Table 1 indicates the results of several similar experiments on other dogs. The slow heart involves a large stroke volume. This, in turn, means that the diastolic size of the heart is large and that the filling

TABLES 1-9

Circulatory constants under experimental conditions

TABLE NUMBER	EXPERIMENTAL CONDITIONS	DOG NUMBER	SURFACE AREA	LESSER CIRCULA- TION TIME	MEAN CIRCULA- TION TIME	TOTAL CIRCULA- TION TIME	PULSE PER MIN- UTE	FLOW PER SQ.M.	"V" PER SQ.M.	SV PER SQ.M.	V/SV
			sq.m.	sec.	sec.	sec.		liters	liters	cc.	
I	Morphine with slow heart	1	0.48	8.5	19.7	19.0	33	3.79	1.355	114.5	11.73
		2	0.43	8.7	19.0	15.5	39	3.47	1.090	88.8	12.70
		3	0.71	4.2	13.5	14.0	51	4.50	1.010	88.0	11.50
		4	0.60	7.2	13.8	12.0	54	4.40	1.085	82.0	13.20
		av.		7.15	16.5	15.1	44.3	4.04	1.135	93.3	12.20
II	Morphine with fast heart	7	0.68	5.9	11.5	11.9	96	6.2	1.19	64.6	18.5
		6	0.44	3.4	8.1	9.8	105	5.5	0.74	52.5	14.1
		av.		4.7	9.8	10.9	101	5.9	0.97	58.6	16.5
III	Morphine and atropine	1	0.48	3.3	7.6	9.7	165	8.26	1.450	44.4	32.7
		2	0.43	3.8	8.7	10.0	93	4.42	0.632	40.7	15.5
		3	0.71	3.2	6.0		120	10.50	1.050	87.5	12.0
		8	0.58	3.0	5.8	6.0	120	10.00	0.950	83.1	11.4
		av.		3.3	7.0	8.6	125	8.30	1.021	63.9	16.0
IV	Morphine, adre- nin 20 seconds before dye	1	0.48	22.0	41.0	20.0	51	1.58	1.08	31.1	34.8
		2	0.43	14.0	26.6	17.0	60	2.37	1.05	39.5	26.6
		3	0.71	19.5	41.6		75	2.92	2.02	38.9	52.4
		6	0.44	19.5	41.0	21.5	57	1.84	1.38	31.8	43.4
		av.		18.8	37.6	19.5	61	2.18	1.38	35.3	39.1
V	Morphine, adre- nin 60 seconds before dye	2	0.41	12.3	35.1	18.2	60	2.04	1.190	33.9	35.1
		5	0.42	14.9	43.3	29.1	54	1.95	1.410	36.2	39.0
		av.		13.6	39.2	23.7	57	2.00	1.300	35.1	37.0
		1	0.48	7.0	14.3	12.0	63	9.85	2.350	156.0	15.1
		6	0.42	6.9	14.5	13.1	69	3.84	0.924	55.5	16.7
		7	0.68	11.3	18.2	10.0	66	5.06	1.590	76.0	20.9
		av.		8.4	15.7	11.7	66	6.25	1.620	95.8	16.9
VI	Morphine-atro- pine, adrenin 20 seconds before dye	5	0.45	7.0	16.3	9.3	165	3.65	0.99	22.0	45.0
		8	0.51	7.2	15.0	10.8	105	3.07	0.77	29.2	26.4
		9	0.70	9.0	23.5	20.0	156	2.30	0.90	14.7	61.2
		av.		7.7	18.3	13.4	142	3.01	0.89	22.0	40.4
VII	Morphine-atro- pine, adrenin 90 seconds before dye	5	0.43	5.7	9.1	5.9	117	4.92	0.753	42.5	17.7
		3	0.71	8.5	13.9	7.8	93	6.00	1.390	64.3	21.6
		av.		7.1	11.5	6.9	105	5.46	1.072	53.4	20.0

TABLES 1-9—*Concluded*

TABLE NUMBER	EXPERIMENTAL CONDITIONS	DOG NUMBER	SURFACE AREA	LESSER CIRCULATION TIME	MEAN CIRCULATION TIME	TOTAL CIRCULATION TIME	PULSE PER MINUTE	FLOW PER SQ.M.	"V" PER SQ.M.	SV PER SQ.M.	V/SV
			sq.m.					liters	liters	cc.	
VIII	Morphine, amyl nitrite 20 seconds before dye	5	0.44	3.7	9.2	9.3	111	5.80	0.889	52.0	17.1
		6	0.44	4.8	9.7	8.3	108	4.59	0.745	42.5	17.5
		8	0.54	6.2	11.5	10.0	90	5.93	1.130	66.0	17.1
		av.	0.47	4.9	10.1	9.2	103	5.44	0.921	53.5	17.2
IX	Dog with A.V. fistula										
	Before fistula	4	0.60	7.2	13.8	12.0	54	4.40	1.085	82.0	13.20
	After A.V. fistula, morphine alone	4	0.58	4.0	10.9	12.0	42	5.60	1.020	133.0	7.67
	Fistula morphine atropine	4	0.60	4.0	7.8	10.0	140	5.95	0.774	42.5	18.20
	Fistula morphine-adrenin 20 seconds before dye	4	0.63	20.5	30.1	15.5	51	2.70	1.350	53.0	25.40
	Fistula morphine-adrenin 60 seconds before dye	4	0.60	14.7	19.6	8.2	90	7.30	2.380	81.2	29.30

pressure on both sides of the heart is increased (6). Since the increased filling pressure of the left heart is communicated through the pulmonary capillaries, they should be stretched and have a relatively large capacity. This is corroborated by the fact that the calculated volume of blood in the heart, lungs and great vessels (V) is relatively large. This, in turn, is derived from, and consistent with, the relatively long mean circulation time.

In table 2 and in figure 2, we have illustrated experiments in which the morphine did not take effect as well as usual. The dogs were restless and the heart more rapid. The excitement induced increased cardiac output, but the rapid heart resulted in a smaller stroke volume. The volume of blood in the heart, lungs and great vessels may be reduced because the rapid heart pumps blood out of the lungs and a lesser filling pressure is necessary with the smaller stroke volume. These factors combined result in decreased circulation times.

In figure 3 and table 3 are illustrated experiments in which the usual dose of morphine was combined with one or two milligrams of atropine—enough to accelerate the heart and eliminate respiratory arrhythmia.

We had expected from previous work (8, 9) that atropine would not

increase markedly the cardiac output. In one case the flow was low (dog 2) with a reduced stroke volume and diminished blood in the heart and lungs (V). In the other three cases there was a large stroke volume in spite of the rapid heart rate. The very short circulation times indicate a reduced or normal central blood volume (V). The variability of cardiac output under these conditions will have to be explained as a result of further work in which other factors are taken into account.

Various scattered observations have been made upon the effects of adrenin and similar drugs (11, 12, 13, 14, 15, 17, 18) upon the heart output and the circulation time. Here the attempt is made to correlate the effect of adrenin upon the several aspects of hemodynamics in the single animal.

When 0.5 mgm. adrenin is given intravenously 20 seconds before the experiment is made we have concentration curves which are strikingly like those in cases of decompensated heart disease. A typical curve is given in figure 4 and the results of the experiments are summarized in table 4. There is intense vasoconstriction, high arterial pressure, and a slow heart due to the depressor reflexes emanating from the aortic arch and the carotid sinus. The peripheral constriction serves to markedly reduce the cardiac output. This occurs in spite of the fact that the blood pressure is high and the heart is working under considerable strain. Although the heart is slow the reduced output gives rise to a low stroke volume. That the diastolic size of the heart, under these conditions, is increased can be deduced from the fact that the volume of actively circulating blood in the heart, lungs and great vessels is relatively large. This large V indicates increased intrapulmonary pressure and, in combination with the low flow, gives rise to the tentative hypothesis that there may be blood completely stagnating in the pulmonary tree unmixed with dye and hence not included in V. This should be added to V if we are to regard this quantity as an emergency reserve (6).

These experiments are of particular interest in relation to the results of similar experiments performed on cases of cardiac decompensation. In such cases, as well as in those under consideration here, the stroke volume is small, the diastolic size and quantity V large, and the circulation times markedly increased. In both types of case, the time concentration curve of the arterial dye is low and delayed. The ratio, that is, the number of beats necessary to clear the lungs and great vessels of blood, is in each case markedly increased. In both cases the inference is working under conditions which impose labors that are beyond the physiological limits of compensation. In heart disease the condition is relatively permanent and is due to myocardial weakness. In the other case, the condition is temporary and is due to the sudden increase in blood pressure and its reflex effects.

If we are justified in saying that during the activity of the depressor mechanism, the animal has mobilized its cardiovascular reserves for the

emergencies consequent upon the situation which causes these changes, we can postulate that the damming back of blood in the lungs is an important phase of the process. The next step in the carrying out of this process is indicated by the following experiments:

In table 5 and figure 5 experiments are illustrated in which the determinations were made 60 seconds after the injection of 0.5 mgm. of adrenin. In some of these experiments (dogs 2 and 5) the condition is exactly as it was in the previous experiments. The heart is slow, circulation times long and the curve flat and delayed, i.e., the cardiac mechanisms are unable to compensate. In other cases the depressor mechanism has let go, vasodilatation has evidently reduced the blood pressure and increased the flow, as has been shown to be the case when small doses of adrenin are given (12, 17, 18). The changes in V are correlated with the increased flow and increased stroke volume. This latter factor results in increased left ventricular filling pressure and pulmonary dilatation, and further, the increased flow might be expected to pick up and carry on, mixing with the dye, such stagnant blood as would exist in the lungs during the activity of the depressor mechanism. These factors together tend to reduce the circulation times and have a parallel in a similar set of conditions which exists during recovery from cardiac decompensation where the increased flow and reduced circulation times occur in spite of an increase in volume of the actively circulating blood in the heart, lungs and great vessels.

In order to find out whether the peripheral vasoconstriction or the slow heart rate immediately following adrenin, is responsible for the reduced cardiac output and other changes in the circulation, it was thought well to repeat the experiments with adrenin, on dogs which were morphinized and atropinized as well.

As is seen in table 6 and figure 6, the output is well below normal in spite of the rapid heart. The volume of actively circulating blood in the heart, lungs and great vessels, on the other hand, is reduced, giving circulation times well below the morphine-adrenin experiments. These factors are correlated with the markedly reduced stroke volume and serve to indicate whereas the volume flow of blood through the circulation is regulated peripherally, the degree of congestion in the heart, lungs and great vessels and hence in part the circulation times depend upon the activity (rate) of the heart.

There is an apparent contradiction here with the situation in the decompensated cardiac cases where, in spite of the rapid heart, the amount of blood in the heart, lungs and great vessels is markedly increased. This is to be explained by the fact that the diastolic size and filling pressure is in the one case (cardiac) high and in the other case low. In other words, the atropinized dog's heart, even under the influence of adrenin, is competent to pump blood into the circulation, whereas the heart of the de-

compensated cardiac can do so only under the stimulus of enough stretching to involve a high filling pressure and marked pulmonary congestion. Atropine helps to compensate the normal heart to the task set by adrenin but could not be expected to help compensate the weakened heart to the task set by heart disease.

If the experiment is performed 90 seconds after the adrenin is given, the atropinized dog responds with an increased flow, "V," and stroke volume. The peripheral constriction has abated and the heart is being regulated accordingly (see table 7).

In order to observe the effect of an opposite set of conditions, the dogs were given amyl nitrite, one ampoule, by inhalation. The experiment was performed 20 seconds later. The results are seen in table 8 and figure 7. This drug increases the heart rate and causes a marked reduction in the blood pressure through its effect of relaxing the arterioles. The flow per minute is increased perhaps significantly above the normal. The markedly reduced circulation times give rise to the conclusion that, under these conditions, the heart is able to reduce the central congestion (V) well below ordinary limits. In other words, the heart pumps the blood out of the lesser circulation and into the greater.

A similar set of conditions is involved when an arteriovenous fistula is made connecting large vessels. Prof. C. E. Bird has kindly made a fistula between the femoral artery and vein of dog 4, and allowed us to experiment with the animal. The results of these experiments are given in table 9. In the first experiment under morphine, the heart is slow and the output increased (see dog 4, table 1). The quicker circulation times leave the quantity V very similar to that found in the normal dog under morphine. When the dog with the fistula is given morphine and atropine, the heart rate is greatly increased but there is no marked increase in output. Circulation times are reduced and there is a decrease in the quantity V. The situation as a whole is almost, if not quite parallel to the situation in the normal dog after amyl nitrite.

When the dog with the fistula is given the usual dose of adrenin, the change is very similar to that produced by adrenin in the normal dog. The greatest differences are traceable directly to the fistula and involve increases in flow and in the quantity V.

It is a pleasure to recognize the technical assistance of Mr. J. H. Rompf.

SUMMARY

1. An analysis is attempted, by means of the injection method, of the relations between the cardiac output, the circulation time and the volume of actively circulating blood in the heart, lungs and great vessels, in response to cardiovascular drugs and the arteriovenous fistula.

2. Morphine slows the velocity of the circulation, increases the central blood volume and probably leaves unchanged the volume flow.

3. Atropine increases the velocity of the circulation and either increases the volume flow or leaves it unchanged. In the latter case there is a marked reduction in the central volume of active blood.

4. Adrenin at first (depressor effect) reduces both the velocity and the volume flow to such a degree that the findings are similar to those in decompensated heart disease. As the effect of the drug wears off, the volume flow and velocity are both markedly increased.

5. The fact that after atropine, adrenin causes a reduced flow in spite of the rapid heart, but gives a small central blood volume and hastens the velocity of the circulation, indicates that the volume flow is to a great extent a function of peripheral constriction and that the central active blood volume and hence, in part, the circulation times are a function of the heart's activity (rate).

6. Amyl nitrite increases the flow and the velocity of the circulation. Under this drug and with the A.V. fistula as well, the heart pumps blood from the lesser into the greater circulations, giving a markedly reduced central active blood volume.

BIBLIOGRAPHY

- (1) HAMILTON, W. F., J. W. MOORE, J. M. KINSMAN AND R. G. SPURLING. *This Journal*, 1928, lxxxiv, 338.
- (2) HAMILTON, W. F., J. W. MOORE, J. M. KINSMAN AND R. G. SPURLING. *This Journal*, 1928, lxxxv, 377.
- (3) MOORE, J. W., J. M. KINSMAN AND W. F. HAMILTON. *This Journal*, 1929, lxxxix, 331.
- (4) KINSMAN, J. M., J. W. MOORE AND W. F. HAMILTON. *This Journal*, 1929, lxxxix, 322.
- (5) HAMILTON, W. F., J. W. MOORE, J. M. KINSMAN AND R. G. SPURLING. *This Journal*, 1930, xciii, 654.
- (6) HAMILTON, W. F., J. W. MOORE, J. M. KINSMAN AND R. G. SPURLING. *This Journal*, 1932, xcix, 534.
- (7) COWGILL, G. R. AND D. L. DRABKIN. *This Journal*, 1926, lxxxi, 36.
- (8) MARSHALL, E. K., JR. *Journ. Pharm. Exper. Therap.*, 1926, xxix, 167.
- (9) TAPPAN, V. AND E. H. TORREY. *This Journal*, 1926, lxxviii, 376.
- (10) SMITH, W. C., C. S. BURWELL AND M. J. DEVITO. *Journ. Clin. Invest.*, 1928, vi, 237.
- (11) GROSS, R. E. AND R. MITTERMAIER. *Pflüger's Arch.*, 1926, ccxii, 136.
- (12) ROMM, S. O. *Pflüger's Arch.*, 1924, cciv, 668.
- (13) EDMUNDS, C. W. *This Journal*, 1907, xviii, 129.
- (14) REYNOLDS, C. AND S. N. BLACKBERG. *Proc. Soc. Exper. Biol. and Med.*, 1927, xxiv, 871.
- (15) LANGLOIS, P. AND G. DESBOUIS. *Journ. physiol. et pathol. gen.*, 1912, xiv, 282.
- (16) HAMILTON, W. F. AND A. B. MORGAN. *This Journal*, 1932, xcix, 526.
- (17) v. EULER, U. AND G. LILJESTRAND. *Skand. Arch. f. physiol.*, 1927, lii, 243.
- (18) ODAIRA, T. *Tohoku Journ. Exper. Med.*, 1925, vi, 325.

MOVEMENTS OF THE BASE OF THE VENTRICLE AND THE RELATIVE CONSTANCY OF THE CARDIAC VOLUME

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The view that the heart remains relatively constant in volume in diastole as in systole, has been maintained by one of us on the basis of an analysis of the cardiopneumogram (1). Thus cardiac volume changes produce pressure changes within the lungs which, when transmitted through the respiratory tree and corrected for the elastic give of the chest walls, seem to be produced by a cardiac volume change of 1 or 2 cc., (certainly less than 7.5 cc.) and last through only a part of systole. This has been confirmed in part by E. Holzlöhner (2) who admits that the net systolic decrease in intrathoracic blood volume is small (5 cc.) but insists that much larger quantities of pulmonary air (25-30 cc.) are displaced by changes in intrathoracic blood volume during the course of systole. That the quantities of air which move in and out of the chest during the cardiac cycle are of the order of 1 or 2 cc., is easily demonstrated by a simple experiment. If one fills the mouth with smoke and maintains respiratory standstill with the internal nares closed and the glottis open, a tiny puff of smoke is seen to issue from the lips toward the end of each ventricular systole. The amount of this is usually not more than 0.5 cc. as contrasted with Holzlöhner's figure of 25 to 30 cc.

Teleologically one might well expect that the heart is organized so as to move blood and blood alone. It does not waste energy moving the contiguous tissues about. The heart is able to pump blood without much displacement of contiguous tissues through the fact that its major pumping action is due to the caudo-cephalad movement of the atrioventricular septum. This in turn insures the reciprocal action of the two chambers so that the auricles fill during ventricular systole, and ventricular filling occurs at the expense of a reduction in the volume of the auricle.

It has long been known that the base of the ventricle makes a large excursion toward the apex during systole and that the apex makes little move-

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This paper is from a thesis submitted by Mr. Rompf in partial fulfilment of the requirements for the Master's degree at the University of Louisville.

ment at any time (Da Vinci, 3). Though the truth of these observations has not been questioned, their significance in regard to the time of auricular filling has not been fully appreciated.

Movements of the base of the heart. In order to make further inquiry into the nature of the cardiac movements, records were taken of the caudo-cephalad movements of the base and apex of the ventricle in the frog, turtle and dog.

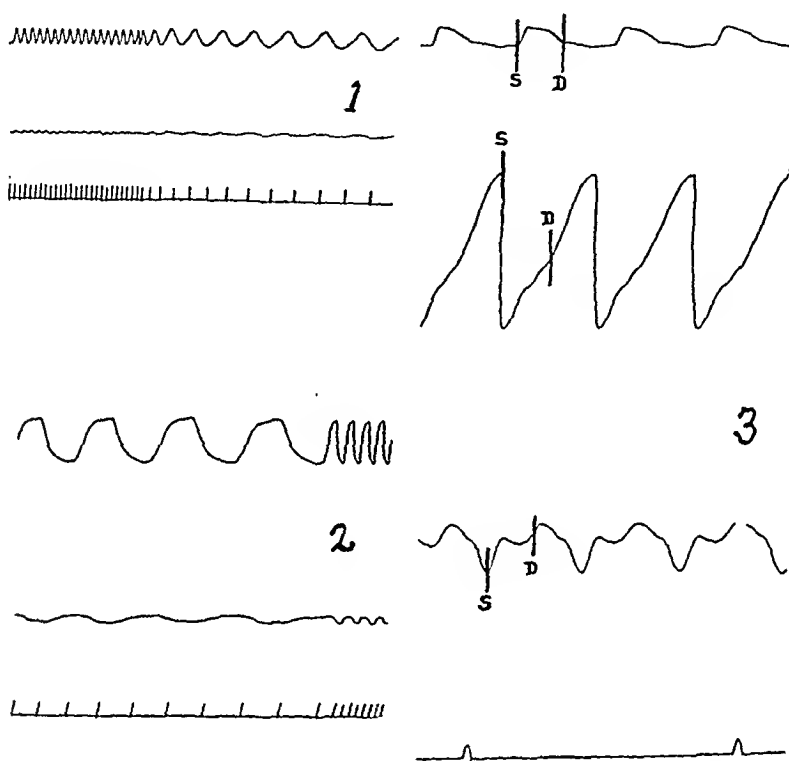


Fig. 1. Mechanically-recorded graphs of the movement of the ventricular base of the frog's heart, upper curve, and of the ventricular apex, middle curve. Lower curve, time in seconds. Downstroke indicates a caudad movement. Magnification of levers is 2:1.

Fig. 2. The same as figure 1—turtle heart.

Fig. 3. Dog heart similar to figure 1. Upper curve, pulse; second curve, movements of ventricular base; third curve, movements of ventricular apex; fourth curve, time in seconds.

The movements were recorded by means of light, delicately counterpoised L-shaped balsa-wood levers. On the end of the upright arm a small barbed hook was pinioned so that it could be thrust into the A.V. groove or into the apex of the heart in such a way as to record only cephalo-caudad movements. The records were taken on cellophane, smoked with a kerosene lamp and fixed in thin shellac (cf. 4). They could be printed photographically with great ease and clearness.

As seen in figures 1, 2 and 3, in all three animals the base of the heart moves downward toward the apex during systole and the apex makes very slight movements. The frenulum which is more or less directly anchored to the anterior abdominal wall, plays an important part in fixing the apex of the turtle heart, for when it is cut the movements of the apex are greatly increased and those of the base greatly decreased. The frenulum of the bullfrog plays a much less important rôle since cutting this strand of connective tissue which is sometimes attached to the posterior surface rather than to the apex, causes little change in the normal movements of the heart.

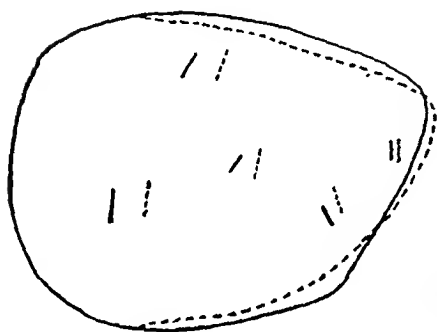


Fig. 4

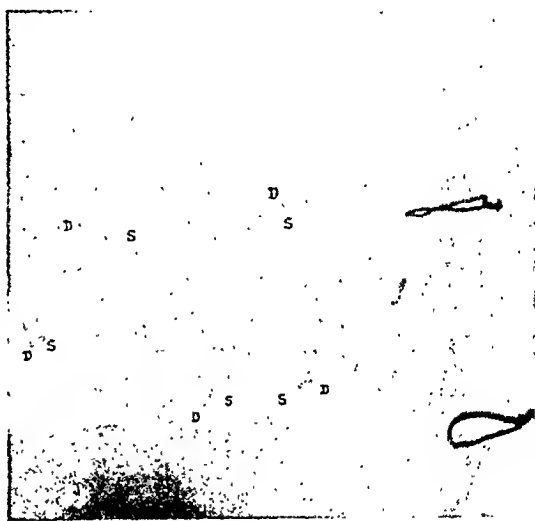


Fig. 5

Fig. 4. Fluoroscopic tracing of diastolic (solid line) and systolic (dotted line) portions of the cardiac outline and of metallic objects placed in the ventricular myocardium. Healthy intact dog.

Fig. 5. X-ray picture of the same heart as figure 4, from a slightly different angle. The diastolic positions of the metallic objects are marked *D* and the systolic positions are marked *S*.

The dog has of course no frenulum. The forces which hold the apex stationary (in this dimension) have been analyzed (1). The most important of these perhaps is the recoil of the heart against the aortic pressure.

That these movements occur not only when the heart is exposed but also when it is in its normal relations in the thorax, is shown by visualizing the base and apex of the ventricle fluoroscopically. To do this the shaft of the barbed point of a fish-hook was inserted into a long needle. The needle was thrust through the chest wall of an etherized dog and under the fluoroscope the barb was dislodged in the myocardium at the base or apex of the ventricle. In other experiments the chest was opened aseptically and bits of silver wire were placed in the ventricular wall near the A.V. groove as well as near the apex.

When the animals had recovered from the immediate effects of these procedures they were perfectly normal, active and playful. With the dog on its side the fluoroscopic appearance was remarkable. To one who had been in the habit of thinking of the cardiac contractions in terms of the small undulations of the edges of the ventricular shadow, the movements of these foreign bodies seemed fantastically vigorous. Only their constancy from day to day, their regularity and the autopsy findings dispelled the notion that the metal objects were not washing about in the cardiac cavities.

As seen from the outline sketch (fig. 4) and the skiagram (fig. 5), the foreign objects approach the apex of the heart during each systole. The distance of approach is roughly proportional to the length of muscle between the object and the apex. The movements toward the apex are much greater than those toward the axis.

These findings emphasize the doubt cast upon the validity of cardiac output determinations derived from calculations which have as their basis the difference in area of the systolic and diastolic x-ray heart shadows (5, 6). The atria cannot be distinguished from the ventricles by x-ray. Therefore, the x-ray method gives us only an indication of the total heart volume change and not the volume change of the ventricles as would be needed for output determination. In order to determine the output of the heart by x-ray, it is necessary to visualize the movements of the top as well as of the sides of the ventricles. We might hazard the guess that calculations which take into account the shortening of the ventricle, the consequent thickening of its walls as well as their inward movement, would be rather complex. Bardeen's formula which is based upon measurement of the whole heart, is hardly applicable to such calculations.

Volume changes of auricles and ventricles. If the anterior body wall of a normal frog is carefully observed a slight pulsation can be seen. This slight movement is by no means comparable to the volume of blood which is forced from the heart at each beat. The tissues forming the body wall in front of the heart are so thin and flexible that one would expect them to follow changes in the cardiac volume. Since the movements impressed upon the anterior body wall by the heart are barely visible, one is led to the conclusion that the total heart volume remains nearly constant during the cardiac cycle.

When the heart is exposed a movement of the organs surrounding it would be observable at each systole if this were not the case. No such movements can be seen because the downward movement of the A.V. septum at each systole allows the auricle to fill during this period. The heart is thus enabled to expend its energy in moving blood instead of wasting some of it in moving the surrounding organs.

Evidence to substantiate this attitude was obtained from simultaneous

records of the volume changes of auricle and ventricle in the frog and from similar experiments on the turtle and dog heart.

The apparatus consisted of very delicate tambours which could record volume changes from glass cardiometers or from the pericardial cavity by

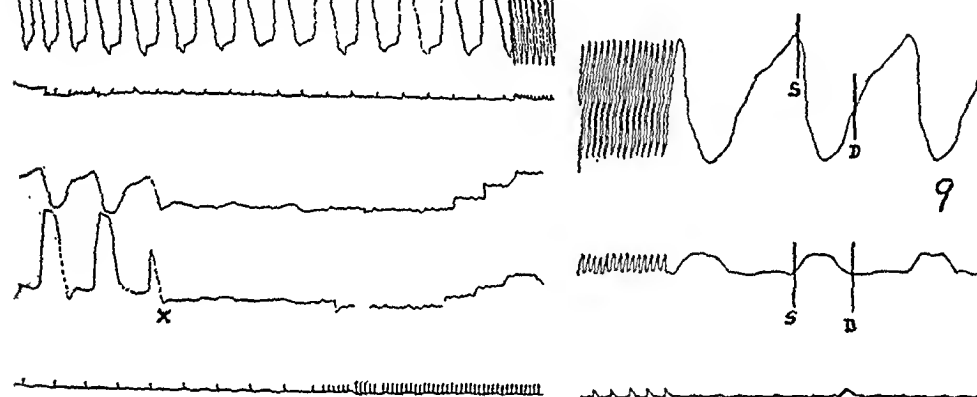
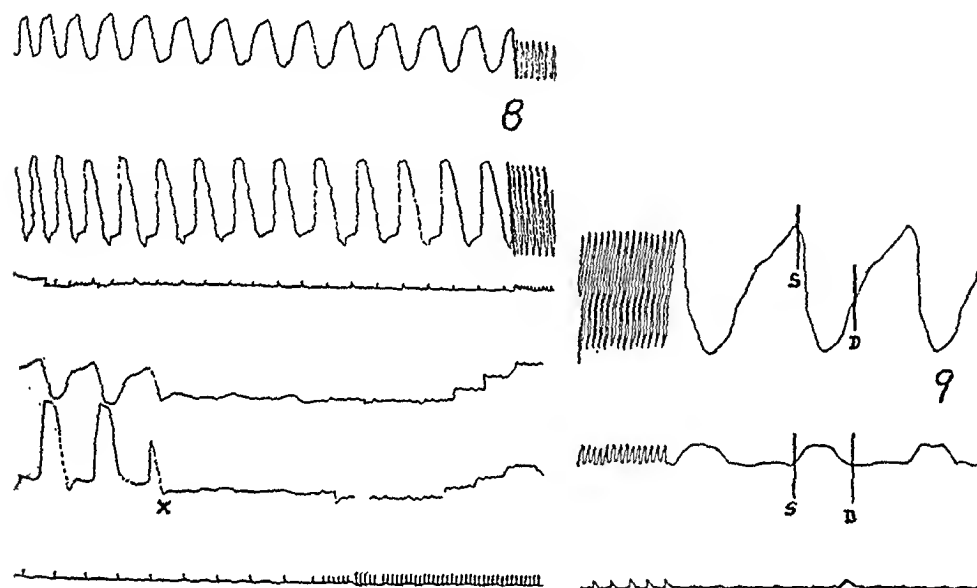
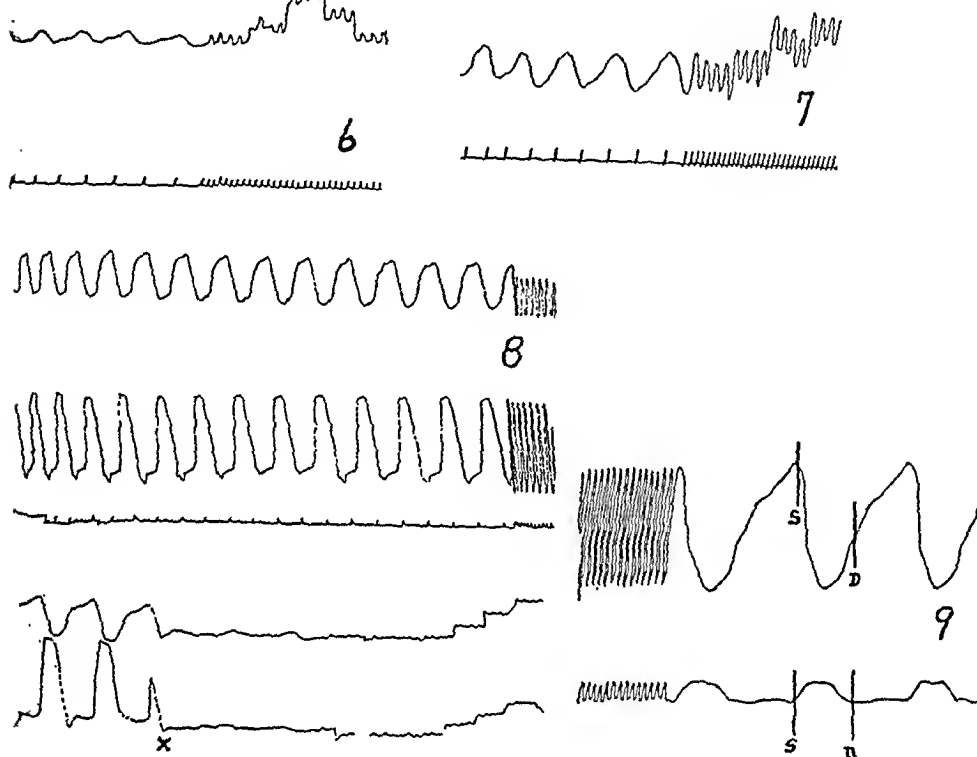
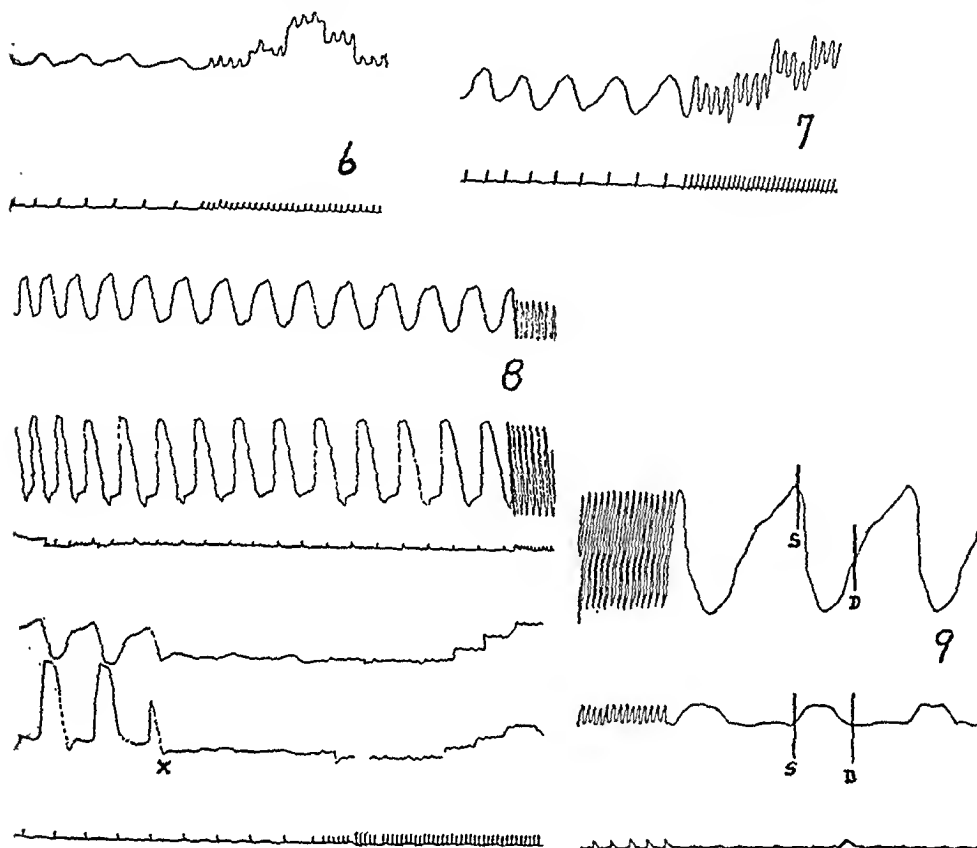


Fig. 6. Volume changes of the frog's heart as recorded from the pericardium. The "staircase" appearance is the calibration where 0.1 cc. portions of air were successively injected into the system and withdrawn. Time in seconds.

Fig. 7. Frog heart, cardiometer cup over the whole heart. Calibration as in figure 6. Time in seconds.

Fig. 8. Volume changes of the auricle, upper curve, and ventricle, middle curve, taken simultaneously. At X a tube connecting the two recording systems was opened. Calibration as in figures 6 and 7. The "steps" indicate about 0.05 cc. for each recorder. Time in seconds.

Fig. 9. Upper tracing, oscillations of pressure within the dog's pericardium (see text); middle tracing, pulse; lower tracing, time in seconds.

direct air transmission. A water manometer was connected into the system and pressure was maintained at a level lower than 2 mm. of water. The records could be quantitated by injecting, with a syringe, small quantities of air as they were being taken.

When the tambour was connected to record volume changes of the frog or turtle heart within the pericardial cavity, net changes were small—less than 0.1 cc. (see fig. 6). They were of the same order when a glass cardiometer was placed over the whole heart (fig. 7), but when separate cardiometers were placed over auricle and ventricle, the volume changes of each chamber were five to ten times as great (fig. 8). Furthermore, they were reciprocal, that is, when the auricles emptied the ventricles filled and *vice versa*. This is shown not only by careful examination of the records, but also by the results of opening a cross connecting tube which allowed air to flow from one recording system into the other.

We were unable to get simultaneous records of the auricle and ventricle in the turtle. However, by comparing the ventricular volume change (0.7 cc.) with that of the whole heart (0.1–0.05 cc.) it is easily seen that the auricle must be filling as the ventricle empties and *vice versa*. Due to the fact that the manipulations involved in these experiments on the turtle and frog probably interfere more with venous inflow than with arterial outflow, one is justified in assuming that in the intact animal the auricular filling would be more prompt and complete and would hence compensate even more closely the ventricular emptying.

A few attempts were made to repeat Stefani's (7) experiments in which he recorded the pressure changes within the partially inflated dog's pericardial cavity. The chest was open and the pressure and anatomical relationships which give rise to the normal auricular filling, were quite disarranged. Further, mechanical oscillations of the heart within the flexible pericardial cavity gave rise to oscillations of pressure which could not be rightly attributed to cardiac volume changes.

We did, however, obtain records which were closely like those of Stefani. Due to the fact that we took simultaneous pulse records we came to the conclusion that the rapid inflow phase (fig. 9) which Stefani attributes to "active diastole," really occurs, in part at least, during systole. If this is in fact a reflection of a true cardiac volume change, it seems reasonable to attribute it to auricular filling during systole. This is quite consistent with the fact that the auricular pressure is lowest in early systole (8), as well as with the fact that Burton-Opitz (9) observes a marked increase of jugular flow during ventricular systole.

SUMMARY

1. Direct recording of the movements of the base and apex of the heart of the frog, turtle and dog shows that at each systole the base of the heart makes a large movement toward the apex, while the apex remains almost stationary. This enables the heart to expend its total energy in moving blood instead of wasting part of it in moving the surrounding organs.

2. The same thing is shown in the normal intact dog when the base of

the ventricle is visualized fluoroscopically by means of small metal objects placed in the myocardium near the A.V. groove.

3. When volume changes of the frog's auricle and ventricle are recorded separately, they are seen to be reciprocal, the auricle fills as the ventricle empties and *vice versa*. Thus volume changes of the heart as a whole are very small.

4. Similar experiments on the turtle and dog indicate similar conclusions.

BIBLIOGRAPHY

- (1) HAMILTON, W. F. This Journal, 1930, xci, 712.
- (2) HOLZLÖHNER, V. E. Zeitschr. f. Biol., 1932, xcii, 293.
- (3) STARLING, E. H. Human physiology, 1930, p. 722. Lea & Febiger, Philadelphia.
- (4) VISSCHER, M. B., W. F. WICHART AND T. H. THIENES. Science, 1931, lxxiii, 99.
- (5) MEEK, W. J. AND J. A. E. EYSTER. This Journal, 1923, lxiii, 400.
- (6) HODGES, F. J. Radiology, 1928, x, 122.
- (7) LUCIANI, L. Human physiology, 1911, i, 216. English transl., Macmillan & Co., Co., London.
- (8) WIGGERS, C. J. Pressure pulses in the cardiovascular system. 1928, p. 32. Longmans, Green & Co., New York.
- (9) BURTON-OPITZ, R. This Journal, 1902, vii, 435.

STUDIES IN THE B VITAMINS

I. STATISTICAL COMPARISON OF SMALL AND LARGE LITTERS OF RATS ON A NORMAL STOCK DIET

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The work of this laboratory for the past several years has been concerned with the effects of nutritional deficiencies on reproduction, pregnancy, lactation, growth, and pathological conditions in the albino rat. For this reason it has been highly important to standardize carefully the stock litters from which our experimental animals are obtained. In previous years, in this, as in many other laboratories, six young per litter was considered a "normal" number. To assure comparative growth results and in no case to subject the nursing mother to undue strain, all litters of larger size were reduced to six young. In any controlled series of tests litter mates are preferable to less closely related individuals even in a highly inbred stock. It was therefore determined to study carefully the individuals of larger litters to see if experimental accuracy demanded the waste of animals entailed in the reduction of litters.

The problems presenting themselves were: 1, the obtaining of consistently large litters by careful selective breeding, and 2, the securing of data on *a*, comparative weight changes of mothers during lactation of large and small litters; *b*, comparative birth weights of young in large and small litters; *c*, comparative weaning weights of young in large and small litters; *d*, comparative weaning weights of young in large litters and those in large litters reduced in number early in the lactation period; *e*, comparative mortality in large and small litters.

This investigation covered a period of five years, and our statistics are compiled from records on 1,624 young normally born on general stock diet.

The stock of the laboratory was a mixture of a Portland albino rat strain with fifteen animals obtained from the Evan's laboratory at the University of California. In 1926, a few months before this investigation was begun, six rats were procured from the Johns Hopkins laboratory for interbreeding. At present we are able to trace the heredity of any given rat back to these original individuals.

Our animals are hardy, active, and tame. They are housed in large sawdust-bottomed cages that are well lighted, well ventilated, and clean. The diet consists of McCollum's grain mixture¹ using whole grain products, fresh minced vegetables, whole milk, and a small portion of table scraps. Cod liver oil² and iodine salts are supplied in addition to this diet.

To increase the size of litters our breeding animals were selected only from litters of nine or more young which showed approximately equal numbers of males and females. Only the most nearly perfect animals were chosen. The mothers were allowed to nurse their entire litters regardless of size for twenty-eight days. Within two years the mean number of young per litter was 9.26 as compared to 7.07 young per litter for the preceding year and a half, a significant increase. Following this period intensive selective breeding was discontinued, but the stock already obtained

TABLE 1
Size of litters, 1926-1931

YEARS	NUMBER OF LITTERS	NUMBER OF YOUNG	RANGE IN NUMBER OF YOUNG	MEAN NUMBER OF YOUNG	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE	DIFFERENCE OF MEANS
							STANDARD DEVIATION	PROBABLE ERROR ² dif.
1926-1927	30	212	3-11	7.07	±0.27	±2.18	4.13	
1928-1929	72	667	2-14	9.26	±0.20	±2.50	5.20	6.64
1930-1931	87	745	2-16	8.65	±0.19	±2.60	5.77	4.79
1928-1931	159	1,412	2-16	8.99	±0.14	±2.59	5.79	5.27
1926-1931	189	1,624	2-16	8.59	±0.13	±2.61	5.62	5.07

Selective breeding of rats carried on in 1928-1929 produced the significant increase in the mean size of litters noted above. The decline in the mean for 1930-1931, when careful selection was discontinued, is interesting but cannot be considered significant. The final effect shown is a distinct change in the strain after 1927 when any of the year groups are compared.

was closely inbred. There was a subsequent decrease to 8.65 young per litter in the year and a half following. (See table 1.)

To determine whether the nursing of large litters placed undue strain on the mothers, careful weight records of the females during lactation were kept. Weight changes of the mother during each of the four weeks of lactation were considered indicative of her condition. These weight

¹ McCollum's grain mixture (ground whole grain products):

Wheat.....	1,000 parts
Corn.....	1,000 parts
Oats.....	1,000 parts
Flax seed.....	400 parts
Casein (unpurified).....	400 parts
Calcium carbonate.....	20 parts

² Supplied by Mead, Johnson & Co., Evansville, Indiana.

changes were correlated with the number of young in the litters. Examination of table 2, in which the results are given, will show that the correlations are not high enough to be considered significant in any case. These

TABLE 2

Correlation of weight changes in mothers during lactation with number of young in litters

	NUMBER OF LITTERS	NUMBER OF YOUNG	RANGE	MEAN	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE STANDARD DEVIATION	CORRELATION	PROBABLE ERROR OF CORRELATION
One week:									
Number of young in litters			3-12	7.77	± 0.18	± 2.32	4.31		
	72	567						$+0.15$	± 0.08
Weight changes (grams) in mothers			-40-+35	$+0.07$	± 1.07	± 13.50	5.56		
Two weeks:									
Number of young in litters			3-14	7.65	± 0.20	± 2.38	5.04		
	68	532						$+0.23$	± 0.08
Weight changes in mothers			-50-+25	-4.52	± 1.59	± 19.38	3.87		
Three weeks:									
Number of young in litters			3-14	7.56	± 0.20	± 2.41	4.98		
	63	488						-0.07	± 0.09
Weight changes in mothers			-50-+30	-5.63	± 2.17	± 20.33	3.94		
Four weeks:									
Number of young in litters			3-14	8.16	± 0.23	± 2.40	5.00		
	51	416						-0.06	± 0.09
Weight changes in mothers			-65-+50	-4.39	± 2.15	± 22.76	5.05		

The correlations approach zero and in no case are high enough to be considered significant. These results indicate that large litters do not of themselves subject the nursing mother to undue strain.

results indicate that in our animals the nursing of large litters does not of itself subject the mother to undue strain.

To determine the effect on the young of the production of large or small litters, the weight records from birth to weaning were studied, as were the mortality records for the nursing period (fig. 1.)

TABLE 3

Correlation of weight of young at birth with number of young in litters

	NUMBER OF LITTERS	NUMBER OF YOUNG	RANGE	MEAN	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE STANDARD DEVIATION	CORRELATION	PROBABLE ERROR OF CORRELATION
Number of young in litters			2-16	8.78	± 0.12	± 2.28	6.58		
Birth weight in grams	164	1,440	3.7-6.5	5.27	± 0.03	± 0.58	5.00	-0.21	± 0.05

The negative correlation of 0.21 is small but probably reliable. This indicates that an increase in the size of the litter may tend to be accompanied by a small decrease in birth weight.

TABLE 4

Correlation of weight gains to weaning with number of young in litters

	NUMBER OF LITTERS	NUMBER OF YOUNG	RANGE	MEAN	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE STANDARD DEVIATION	CORRELATION	PROBABLE ERROR OF CORRELATION
I. Using all litters available									
Number of young in litters			1-15	7.79	± 0.26	± 3.10	4.84		
Weight gains (grams), 28 days	67	522	15-59	31.70	± 0.90	± 10.92	4.12	+0.15	0.08
II. Using only large litters and large litters reduced in size early in the lactation period									
Number of young in litters			1-15	8.94	± 0.26	± 2.64	5.68		
Weight gains (grams), 28 days	49	438	15-51	32.45	± 1.03	± 10.71	3.46	+0.21	0.09

In I weight gains in litters of all sizes are considered. In II reduced and unreduced large litters are considered. Both parts of the table show small positive correlations, which, however, cannot be considered significant. These results therefore indicate that young from large litters show growth gains which are entirely comparable to those of young from smaller litters.

The results of the comparison of the average birth weights with the number of young in the litter are given in table 3. A small but probably reliable negative correlation was found, indicating that an increase in the

size of the litter tends to be accompanied by a small decrease in birth weight.

In table 4 are given our statistical data on weight gains to weaning, 28 days. A correlation was first run between the average weight gains and the number of young per litter at weaning on all animals for which records were available up to 1930. (At that time breeding to increase the weights of the individual animals was begun, so the weights for 1930-1931 are not comparable and are not included.) The results showed a small positive correlation, tending to favor the large litters, but too small to be considered significant. These findings indicate that growth in the large litters was definitely normal for our strain.

To see if the young of large litters were being permitted optimal growth, a second correlation was run. Litters of eight or more young were taken, and in about half of these the number of young was reduced early in the

TABLE 5
Mortality

LITTER SIZE	NUMBER OF LITTERS	NUMBER OF YOUNG	MORTALITY NUMBER	MORTALITY PER CENT
Small (1-4 young).....	14	46	8	19.0
Medium (5-8 young).....	68	459	22	4.5
Large (9-12 young).....	101	1,033	43	4.2
Very large (13-16 young).....	6	86	7	8.1

The death rate is lowest in the large and medium-sized litters and increases in both the small and the very large litters in our series.

lactation period. In this correlation (see table 4, II) the gains of reduced litters are no greater than the gains of the unreduced litters.

An examination of our data on mortality of young during the lactation period indicates a tendency toward a reversed curve—a greater death rate in the small and in the very large litters (fig. 1 and table 5).

In the small litters (1 to 4 animals) the number of young may not be sufficient to give an accurate picture of the death rate, and it is not improbable that if the data were increased the percentage of mortality would be somewhat lower. However, in our series these litters commonly showed a poorer type of rat. They were most often produced by second rate animals or from the accidental matings of very young animals. Also, it must be considered that moribund young of larger litters of low vitality might have been eaten by the mother before being found and recorded, thus swelling the number of substandard small litters.

The number of very large litters (13 to 16 animals) is too small to warrant definite conclusions being drawn. However, the chances for exposure and mechanical deaths are undoubtedly increased in these, and it is not unlikely that they would show a higher than average death rate even in a more

extended series. The young of the very large litters are almost always noticeably strong and hardy, so that if part of the litter were given to other mothers the death rate might be considerably reduced.

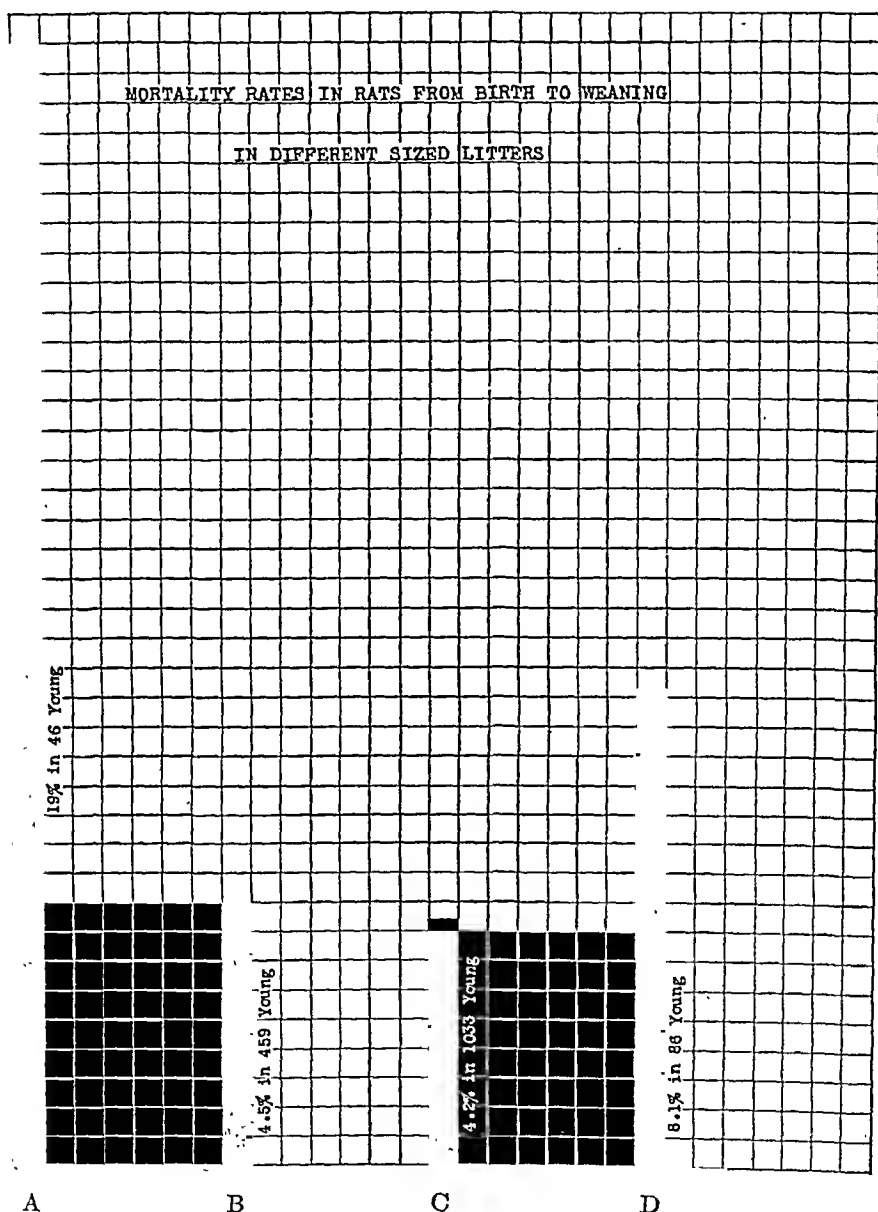


Fig. 1

A—Small: (1-4 young), 14 litters, 46 young

B—Medium: (5-8 young), 68 litters, 459 young

C—Large: (9-12 young), 101 litters, 1033 young

D—Very large: (13-16 young), 6 litters, 86 young

The "large" and "medium" litters had a distinctly lower death-rate than the "small" or the "very large" litters.

Litters of medium size (5 to 8 animals) showed a mortality of 4.5 per cent; large litters (9 to 12 animals), 4.2 per cent. These results clearly indicate that the viability of the young is not decreased by increasing the size of the nursing litter.

SUMMARY AND CONCLUSIONS

1. This investigation covered a period of five years and was made on 1,624 young born on a normal stock diet.
2. Consistently larger litters were produced by careful selective breeding.
3. The strain of large litters on lactating mothers, as evidenced by change in weight of the mother during the lactating period, was not greater than that in the case of smaller litters.
4. An increase in the size of the litter tended to be accompanied by a small decrease in the average birth weight of the young.
5. Weight gains up to weaning were essentially the same in large and small litters.
6. Weight gains up to weaning were essentially the same in large litters unreduced in size as in large litters reduced early in the lactation period.
7. In litters of from 9 to 12 young mortality during lactation was slightly but not appreciably lower than in litters of from 5 to 8 young. Very small and very large litters (1 to 4 and 13 to 16 animals respectively) showed higher death rates.

From these data it is concluded that the young from large litters compare favorably in hardiness and viability with the young from small litters, and that the increased number of littermates obtainable from large litters (up to 13 young per litter) may safely be used in controlled experimental work.

ADDENDA. For those not readily conversant with statistical methods it may be desirable to point out that

1. To be significant the difference between two means under consideration must be at least four times as great as the probable error of their difference.
2. To be significant a correlation coefficient must be at least four times its probable error.
3. If the population on which data are available is fully representative, i.e., if the distribution is normal, then the range divided by the standard deviation should yield a figure approaching six.

BIBLIOGRAPHY

- DONALDSON, H. H. The rat. *Memoirs of Wistar Inst. of Anat. and Biol.* no. 6. ed. 1, 1915; ed. 2, 1924.
- DUNN, H. L. *Physiol. Rev.*, 1929, ix, 275.
- FISHER, R. A. *Statistical methods for research workers.* Oliver & Boyd, London, ed. 3, 1930.
- GARRETT, H. E. *Statistics in psychology and education.* Longmans, Green & Co., New York, 1926.
- JACKSON, C. M. *Arch. Path.*, 1929, vii, 1042; viii, 81, 273.
- MCCOLLUM, E. V. AND N. SIMMONDS. *Newer knowledge of nutrition.* Macmillan Co., New York, ed. 4, 1929.

STUDIES IN THE B VITAMINS

II. STATISTICAL COMPARISON OF RAT LITTERS ON NORMAL STOCK DIETS WITH LITTERS ON SYNTHETIC DIETS CONTAINING VARYING AMOUNTS OF THE VITAMIN B COMPLEX AND COMBINATIONS OF B₁ AND B₂

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The results of total deprivation of the vitamin B complex are so striking as to show sharply in small, critical experiments. The clear clinical symptoms and the rapid cures have become classic. When, however, the study of the vitamin B complex and its components is concerned not with total deprivation but with optimum and less than optimum amounts no sharp lines are discernible. Individual variations are marked. Apparently carefully duplicated experiments often give dissimilar results. Small numbers of animals become undependable.

The purpose of this study was to apply the statistical method of approach to a sufficiently large number of animals born on certain diets partially defective in all or part of the vitamin B complex, and to compare the results with those obtained with rats born on normal stock diets.

In the following paper a comparative study is made of females on stock and on the experimental diets under consideration, and our statistical data obtained during the lactation period on 2,627 young born on five of our diets are reported. These data include a, weight changes in mothers during lactation; b, size of litters; c, average birth weights; d, average weight gains to weaning, and e, mortality of the young during lactation. The diets include normal stock and four synthetic diets of which two supplied the vitamin B complex in the form of dried brewers yeast,¹ respectively 2 per cent and 10 per cent, and two supplied the B₁ and B₂ factors in varying proportions in the form of preparations made by this laboratory. These last two diets contained respectively 10 per cent B₁ plus 2 per cent B₂ and 5 per cent B₁ plus 10 per cent B₂.

A detailed account of our methods of securing the B₁ and B₂ products is given in paper III of this series (1), as is an explanation of the basis of calculating the percentages of the vitamin B factors in the diet.

¹ Northwestern Yeast Co.

Our B₁ (antineuritic, B, or F) factor was obtained by extracting yellow corn meal² with acid 85 per cent alcohol and adsorbing it onto corn dextrin. The B₂ (growth, P-P, or G) factor was made by autoclaving a 20 per cent suspension of yeast in tap water for 3 hours at 15 pounds pressure. For convenience we took 10 grams of basal food per animal per day as a standard from which to calculate percentages of the vitamin factors in the diet. Thus "10 per cent" is not an actual percentage but represents that one gram of the substance dry or in suspension, or the the extract or concentrate of one gram of that substance, was being supplied in measured amounts to each animal each day. All vitamin portions were carefully measured and fed to each animal before the basal food was placed in the cage.

Two years were spent in standardizing the methods of preparation of our B₁ and B₂ products and the percentages for feeding. Standardization was based on the following points. *a.* Amounts of the B₁ or B₂ preparations, separate or combined, necessary to effect cures in rats on diets deficient in these respective vitamin factors. Comparison of these amounts with curative quantities of whole yeast. *b.* Growth curves of animals on diets supplying B₁ or B₂ or combinations of these factors. *c.* Mortality on these diets as compared with that on yeast and on stock diets; and *d.* oestrous cycles of females on experimental and normal diets. Results of this standardization led us to believe that our B₁ product was relatively pure, but that the B₂ preparation probably contained small quantities of B₁ and of some other factor then unknown. The work of Reader (2) as compared to our findings indicates to us that this was probably B₄.

The stock diet consisted of McCollum's grain mixture using whole grain products, fresh minced vegetables, whole milk, and a small portion of table scraps. Cod liver oil³ and iodine salts were supplied in addition. Experimental rats received casein 18, dextrin 78, salt mixture (no. 185 McCollum) 4, and iodine salts. Their vitamin portions, which were fed separately, consisted of 1 cc. of cod liver oil³ per animal per day, 4 drops of wheat germ oil³ (E) per animal per day, and the suitable amount of the B vitamin. The experimental diets varied only in the form and amounts of the vitamin B complex or its components supplied.

Stock rats were kept in large, sawdust-bottomed cages. Experimental animals had cages with wire mesh bottoms. Females were placed in individual cages, segregated before delivery and given shredded paper for nest building. Stock males were used in all matings.

Since the work of this laboratory was primarily concerned with pregnancy and lactation and only secondarily with growth after weaning, many of the standard methods of vitamin determination have proved to be

² Quaker Oats Co.

³ Mead, Johnson Co.

hardly satisfactory for our purposes. The growth curve of a rat transferred from stock to an experimental diet after weaning does not indicate the sexual maturity or potential fecundity of that individual animal. Nor does it show whether delivery and lactation of young are possible. We determined therefore to base our study entirely on young born of mothers raised on the diets in question.

The litters born on the antineuritic factor (B_1) and on the growth factor (B_2) are all first generation young, being first and second litters of mothers transferred from stock to the experimental diets at from two to three months of age. The earliest litters were obtained only after the mothers had been on the diets for over three months.

A study of the oestrous cycles of females on the stock and the experimental diets was made, using Evans' method (3) of microscopic examination of the vaginal smears. Oestrus occurred in our stock females regularly on the average of about every fourth to fifth day (4). The smears were definite and easy to identify, having large numbers of the characteristic oestrous cells predominating. On the experimental diets, however, particularly in those supplying the B_1 and B_2 preparations considerable variation was seen, both as concerned the length and regularity of the oestrous cycle as well as the type of cells occurring in the smear. It was frequently very difficult to determine when true oestrus was present. Typical oestrous cells were not always seen, and when present were usually smaller and more mixed among leucocytic and epithelial elements than when seen in stock vaginal smears. Frequently, nothing but masses of cellular debris was found. We have been led to believe that this may have represented an abnormal oestrous period.

An examination of our smear records showed in the case of animals on the 10 per cent B_1 2 per cent B_2 diet that at the end of one month on the diet the average oestrous cycle was from 5 to 6 days; at the end of six months, from 7 to 8 days. Individual cycles were very irregular. On the 5 per cent B_1 10 per cent B_2 at the end of one month the average oestrous cycle was 5 to 6 days; at the end of six months, 6 to 7 days. These cycles were also irregular. On 2 per cent yeast, after several months on the diet, average oestrous cycles were 6 to 7 days, while on the 10 per cent yeast diets at this time oestrous cycles were approximately normal, being 4 to 5 days but more irregular than in our stock animals.

Our available data on the diets concerning weight changes in mothers during the lactation period are summarized in table 1. The number of females on the B_1 plus B_2 diets producing young which survived the four weeks of nursing was so small that data on their weight changes are not dependable in a statistical study. This is more or less true of the yeast diets, but it seems worth reporting that so far as they may be relied upon they indicate no greater strain on lactating females on two per cent or ten per cent yeast diets than on the normal stock diet.

A comparative study of litter size, given in table 2, shows that the stock litters were significantly larger in number of young than those of any of the synthetic diets with vitamin B added. The diet quality when judged by this standard shows the following order of value, *a*, stock; *b*, 10 per cent yeast; *c*, 5 per cent B₁ 10 per cent B₂; *d*, 10 per cent B₁ 2 per cent B₂; *e*, 2 per cent yeast. However, the differences in litter size among the experimental diets are not large enough to be statistically significant.

Table 3 indicates the comparison of average birth weights. The stock weights are significantly higher than those on any of the experimental

TABLE 1
Weight changes in mothers during lactation

DIET	NUMBER OF MOTHERS	WEIGHT CHANGES RANGE	MEAN WEIGHT CHANGE	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE STANDARD DEVIATION	DIFFERENCE OF MEANS PROBABLE ERROR dif.
<i>One week:</i>							
Stock.....	72	-40-+35	+0.07	±1.07	±13.50	5.36	
2 per cent yeast....	23	-30-+20	-2.17	±1.62	±11.78	4.24	0.66
10 per cent yeast....	22	-25-+30	-0.23	±1.87	±13.01	4.61	0.05
<i>Two weeks:</i>							
Stock.....	68	-50-+25	-4.52	±1.59	±19.38	3.87	
2 per cent yeast....	19	-40-+25	-0.79	±2.38	±15.36	4.23	0.88
10 per cent yeast....	19	-25-+50	-3.42	±2.43	±16.87	4.74	0.26
<i>Three weeks:</i>							
Stock.....	63	-50-+30	-5.63	±2.17	±20.33	3.94	
2 per cent yeast....	20	-50-+25	-7.25	±2.68	±17.79	4.22	0.32
10 per cent yeast....	16	-35-+30	-1.25	±2.35	±16.47	4.26	1.14
<i>Four weeks:</i>							
Stock.....	51	-65-+50	-4.39	±2.15	±22.76	5.05	
2 per cent yeast....	15	-40-+30	-8.00	±3.16	±18.15	3.86	0.64
10 per cent yeast....	14	-60-+40	-8.93	±3.78	±26.31	3.99	0.70

With the small numbers of animals compared, no significant difference was demonstrated in weight changes in mothers during lactation between diets containing yeast as a sole source of vitamin B and stock diet.

diets. The order of highest average weights proved to be *a*, stock; *b*, 10 per cent yeast; *c*, 5 per cent B₁ 10 per cent B₂; *d*, 2 per cent yeast; *e*, 10 per cent B₁ 2 per cent B₂. Among the experimental diets the differences are not large enough to be statistically significant.

A study of the average individual gains in weight during the lactation period on the different diets (table 4) shows that only the 2 per cent yeast diet is significantly below that of the stock at any time. By weaning, at the end of the fourth week, weight gains on 10 per cent yeast, stock, and 5 per cent B₁ 10 per cent B₂ are found to be practically on a par with each other. Ten per cent B₁ 2 per cent B₂ shows a definitely though not sig-

nificantly lower mean, but 2 per cent yeast maintains its consistently low position. It must be considered that all of the experimental diets have

TABLE 2
Comparative size of litters

DIET	NUMBER OF LITTERS	NUMBER OF YOUNG	RANGE IN NUMBER OF YOUNG	MEAN NUMBER OF YOUNG	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE STANDARD DEVIATION	DIFFERENCE OF MEANS PROBABLE ERROR ¹ dif.
Stock (total).....	189	1,624	2-16	8.59	±0.13	±2.59	5.8	
Stock (1928-1929 only)...	72	667	2-14	9.26	±0.20	±2.50	5.2	
2 per cent yeast.....	40	226	1-12	5.65	±0.29	±2.73	4.8	6.3
10 per cent yeast (total)...	57	379	1-11	6.61	±0.19	±2.12	5.2	5.9
(1929 only).....	22	161	3-10	7.23	±0.25	±1.76	4.5	4.3
10 per cent B ₁ 2 per cent B ₂ (1929).....	17	120	2-9	6.64	±0.28	±1.71	4.7	5.1
5 per cent B ₁ 10 per cent B ₂ (1929).....	24	172	5-12	7.17	±0.26	±1.89	4.2	4.4

This table shows that stock animals produced significantly more young per litter than rats on any of the synthetic diets.

Our experiments with 10 per cent B₁ plus 2 per cent B₂ and 5 per cent B₁ plus 10 per cent B₂ diets were carried on in 1929 only. An increase in litter size produced (by selective breeding) at that time has been reported in paper I of this series (4). For purposes of comparison, therefore, stock for 1928 and 1929 and 10 per cent yeast litters for 1929 only are given.

TABLE 3
Average weight of young at birth

DIET	NUMBER OF LITTERS	NUMBER OF YOUNG	WEIGHT RANGE	MEAN WEIGHT	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE STANDARD DEVIATION	DIFFERENCE OF MEANS PROBABLE ERROR ¹ dif.
			grams	grams				
Stock.....	164	1,379	5.7-6.5	5.27	±0.03	±0.58	5.0	
2 per cent yeast.....	33	189	3.0-6.0	4.49	±0.06	±0.55	5.5	7.1
10 per cent yeast.....	54	371	3.0-6.2	4.79	±0.05	±0.60	5.3	5.3
10 per cent B ₁ + 2 per cent B ₂	13	86	3.3-5.1	4.37	±0.11	±0.57	3.3	5.3
5 per cent B ₁ + 10 per cent B ₂	18	135	3.5-5.4	4.64	±0.08	±0.50	3.8	4.8

The mean stock weight is significantly higher than the means of any of the experimental diets.

percentage mortalities almost double those of the stock, and that it is usually the smaller rats that die, thus tending to raise the weight averages.

(However, from 75 per cent to 90 per cent of deaths on all diets come during the first week.) Later growths on the B₁ plus B₂ diets will be reported in paper III of this series (1).

The mortality of young during the lactation period is given in table 5.

TABLE 4
Weight gains by week to weaning

DIET	NUMBER OF LITTERS	NUMBER OF YOUNG	WEIGHT GAIN	MEAN WEIGHT	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	RANGE STANDARD DEVIATION	DIFFERENCE OF MEANS PROBABLE ERROR _{dif.}
			RANGE	GAIN				
			grams	grams				
<i>One week:</i>								
Stock.....	91	708	1-12	6.20	±0.14	±1.99	6.01	
2 per cent yeast.....	22	123	0-9	4.86	±0.27	±1.87	5.35	5.35
10 per cent yeast.....	33	193	2-10	5.49	±0.28	±2.37	3.79	2.28
10 per cent B ₁ + 2 per cent B ₂	9	63	4-9	6.00	±0.38	±1.70	3.53	0.49
5 per cent B ₁ + 10 per cent B ₂	18	111	2-10	5.94	±0.30	±1.90	4.74	0.76
<i>Two weeks:</i>								
Stock.....	87	683	4-26	13.63	±0.27	±3.73	6.17	
2 per cent yeast.....	20	113	6-15	10.50	±0.46	±3.02	3.31	5.92
10 per cent yeast.....	26	131	7-22	13.50	±0.54	±4.08	3.92	0.22
10 per cent B ₁ + 2 per cent B ₂	9	63	5-28	13.67	±1.05	±4.67	5.14	-0.07
5 per cent B ₁ + 10 per cent B ₂	18	111	5-19	12.56	±0.60	±3.79	3.96	1.63
<i>Three weeks:</i>								
Stock.....	79	595	10-40	22.16	±0.43	±5.72	5.25	
2 per cent yeast.....	17	103	8-25	15.29	±0.78	±4.79	3.76	7.50
10 per cent yeast.....	23	107	14-34	20.09	±0.74	±5.25	4.00	1.24
10 per cent B ₁ + 2 per cent B ₂	8	56	15-30	21.13	±1.06	±4.43	3.61	2.32
5 per cent B ₁ + 10 per cent B ₂	14	87	8-30	20.00	±1.14	±6.35	3.62	1.77
<i>Four weeks:</i>								
Stock.....	66	509	15-59	31.72	±0.87	±10.44	4.31	
2 per cent yeast.....	13	72	13-36	23.92	±1.32	±7.06	3.40	4.94
10 per cent yeast.....	20	89	20-56	32.75	±1.42	±9.40	3.85	-0.61
10 per cent B ₁ + 2 per cent B ₂	7	41	18-38	28.71	±1.14	±4.46	4.71	2.10
5 per cent B ₁ + 10 per cent B ₂	10	66	20-40	31.00	±1.30	±6.08	3.45	0.46

Comparison of the weight gains of the young during the lactation period demonstrates a significantly lower gain for the 2 per cent yeast diet than for stock for the four weeks as well as for the shorter periods. The other experimental diets show no significant variation from stock.

The most striking point to be noted is the high death rate on all experimental diets. Since our stock young were raised in sawdust-bottomed cages and the experimental young in wire-bottomed cages, the possibility of exposure being an influencing factor in the mortality of the latter was considered. Fifteen litters on the experimental diets were born and kept in the regulation stock cages. This group showed a somewhat, although not strikingly lower mortality than was usual for the diets. On the other hand, over 100 stock young born and kept in the wire-bottomed cages showed no increase in death rate.

TABLE 5
Mortality during lactation

DIETS	NUMBER OF LITTERS	NUMBER OF YOUNG	PER CENT OF TOTAL DEATHS DURING LACTATION								TOTAL MORTALITY	
			Mortality first week		Mortality second week		Mortality third week		Mortality fourth week		Number	Per cent
			Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent		
Stock.....	189	1,624	66	82.5	4	5.0	7	8.8	3	3.8	80	4.98
2 per cent yeast...	39	327	103	74.1	2	1.4	21	15.1	13	9.3	139	61.2
10 per cent yeast...	57	380	177	85.1	15	7.2	7	3.4	9	4.3	208	54.7
10 per cent B ₁ + 2 per cent B ₂	19	126	57	90.5			3	4.8	3	4.8	63	50.0
5 per cent B ₁ + 10 per cent B ₂	24	172	54	85.7			2	3.2	7	11.1	63	36.8

Consistently high mortalities among the young (36.8 to 61.2 per cent) during the lactation period were found on all of the experimental diets. Mothers on a stock diet lost only 4.98 per cent of their litters.

SUMMARY

1. Statistical studies are reported on 1,624 normal stock young and on 1,003 young born on experimental diets.

2. A comparison of the oestrous cycles in the mothers of these young is made. Among the experimental diets only on 10 per cent yeast does the oestrous cycle remain approximately normal.

3. Stock litters and the litters born on four synthetic diets varying only in vitamin B content are compared.

4. No significantly greater weight changes on the part of the mothers was found in females nursing litters on a 2 per cent and a 10 per cent yeast diet than in stock females.

5. Litter size (mean number of young per litter) was found to decrease progressively in the following order: stock, 10 per cent yeast, 5 per cent B₁ 10 per cent B₂, 10 per cent B₁ 2 per cent B₂, 2 per cent yeast. There was

a significant difference between stock and all the experimental diets. Among the experimental diets, no significant difference was found.

6. The mean average birth weights were found to be progressively lower in the following order: stock, 10 per cent yeast, 5 per cent B₁ 10 per cent B₂, 2 per cent yeast, 10 per cent B₁ 2 per cent B₂. There was a significant difference between the stock and all the experimental diets. Among the experimental diets, no significant difference was found.

7. The comparative average individual weight gains during the lactation period showed considerable variation, with 2 per cent yeast always significantly lower than stock. At weaning (28 days) 10 per cent yeast, stock, and 5 per cent B₁ 10 per cent B₂ were practically equal, with 10 per cent B₁ 2 per cent B₂ considerably but not significantly lower.

8. Very high mortality rates among the young during the first four weeks of life were found on all experimental series. Possibility of exposure in the wire cages being an influencing factor is considered.

BIBLIOGRAPHY

- (1) MOORE, PLYMATE AND ANDREW. *This Journal*, 1932, cii, 581.
- (2) READER, V. *Biochem. Journ.*, 1929, xxiii, 689; 1930, xxiv, 77.
- (3) EVANS. *Journ. Met. Res.*, 1922, i, 319.
- (4) MOORE, PLYMATE, ANDREW AND WHITE. *This Journal*, 1932, cii, 566.

STUDIES IN THE B VITAMINS

III. EVIDENCE OF A THIRD VITAMIN B FACTOR IN YEAST (B_4) AS SHOWN BY GROWTH CURVES AND CLINICAL SYMPTOMS OF FIRST AND SECOND LITTER YOUNG OF MOTHERS RAISED ON SYNTHETIC B_1 AND B_2 DIETS

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In attempts to analyze the clinical effects of the B_1 and B_2 factors of the vitamin B complex, this laboratory has considered it advisable to use, if possible, animals other than those just weaned from stock diets. By so doing we hoped to eliminate such effects as might be associated with a possible storage of vitamin factors supplied to our normal stock animals (1). It was desirable to secure such synthetic diets, supplying the B_1 and B_2 factors, as would permit our experimental animals to mature, reproduce, and nurse their young.

Since our main interest was to study clinical effects, we sought in our preparing of the B_1 and B_2 factors to establish standardized methods of securing products which, though not necessarily pure in the respective vitamin factors, would be of high enough concentration to maintain our animals approximately normally for several months.

At the time this series of experiments was started (1929) our B_1 and B_2 preparations had been carefully standardized. Certain discrepancies in our findings and the work of others (2) had led us to suspect the presence in yeast of at least one other factor of the vitamin B complex. In our preliminary work on the preparation of a suitable B_2 product (1928) using neutral suspensions of yeast¹ autoclaved over periods varying from 1 to 10 hours at 15 pounds pressure, we found that autoclaving for more than three hours gave us products which were increasingly less satisfactory for the maintenance of our experimental animals to maturity. We therefore adopted as our B_2 preparation a 20 per cent suspension of yeast in tap water autoclaved for 3 hours at 15 pounds pressure, stored in refrigeration until used. (A two week's supply was autoclaved at one time.) Our standardization of the potency of this product led us to believe that it

¹ Northwestern Yeast Co.

probably contained small amounts of the B_1 factor and also of some other factor then unknown, but that it was eminently satisfactory for our purpose of maintaining animals to maturity on synthetic B_1 and B_2 diets. (The work of Reader (3) indicates to us that we were probably dealing with B_4 .) A carefully standardized routine of preparation was established in order to maintain the amounts of B_2 as well as of the unknown substances in our product always at a constant level.

As a source of B_1 we chose an extract of yellow corn meal² made by macerating the meal with 85 per cent alcohol with 1 per cent HCl for one week, pressing out the fluid with a fruit press, washing with fresh acid 85 per cent alcohol, and filtering the mixed alcohols to free them from small corn meal particles. Most of the alcohol was evaporated off by heating 125 cc. portions for 15 minutes in a boiling bath, using a suction condenser apparatus. Small quantities of the fluid were taken to avoid subjecting the vitamin factor to prolonged heating. The concentrated extract was chilled, the protein precipitated by bringing the solution approximately to the iso-electric point by stirring in dilute NaOH, and filtered. The filtrate was adsorbed on white corn dextrin and dried by electric fan at room temperature. The resultant material, of which 1 gram was equivalent to 5 grams corn meal, was powdered, stored in a cool place, and fed daily in accurately measured amounts. This concentrate appeared to be relatively free of the B_2 factor. We had no indications that another factor might be present.

The food of our synthetic diets consisted of casein 18, dextrin 78, salt mixture (no. 185 McCollum) 4, and iodine salts. In addition, each of the experimental animals received as a daily portion 1 cc. cod liver oil³ (vitamins A and D) and 4 drops wheat germ oil (vitamin E). The entire vitamin portion, including adequate, measured amounts of the B_1 and B_2 preparations, depending upon the diet, was carefully measured out and served in a small dish before the basal food was placed in the cage. In this way it was possible accurately to control the vitamin intake of each animal. The diets of the different experimental groups varied only in the amounts of B_1 and B_2 supplied.

It had been determined in our laboratory that approximately 10 grams of basal food per animal per day was a generous allowance, and for the sake of brevity and clearness this amount was adopted as a standard on which to calculate diets. Thus "10 per cent" of any substance is not an actual percentage in the diet, but means that 1 gram of that substance, dry or in suspension, or the extract or concentrate of 1 gram of that substance was being supplied to each animal each day. (In the case of our antineuritic concentrate we used as 10 per cent the extract of 1.2 gram of corn meal to allow for possible loss of the B_1 factor during preparation of the concentrate.)

² Quaker Oats yellow corn meal.

³ Mead, Johnson Co.

On May 1, 1929, two new experimental diets, CXIX and CXX were started. The diets were as given above, with the addition of: in group CXIX, 10 per cent B_1 2 per cent B_2 (0.25 gm. corn meal concentrate and 1 cc. 20 per cent suspension autoclaved yeast); and in group CXX, 5 per cent B_1 10 per cent B_2 (0.13 gm. corn meal concentrate and 5 cc. 20 per cent suspension autoclaved yeast). Hereafter these diets will be referred to simply as CXIX and CXX respectively. A group of 12 normal stock females ranging in age from 70 to 90 days was placed on each of these diets. They appeared to develop normally.

The vitamin proportions here used were based on results of our preliminary work in which it appeared that 10 per cent B_1 or 10 per cent B_2 were the maximum quantities necessary for optimum development of experimental animals taken from stock. These amounts had proved to be effective dosages for the treatment of animals suffering from a deficiency of one or the other vitamin factors.

The animals were kept in large screen-bottomed cages (4 meshes per inch). They continued to gain weight and appeared in excellent condition throughout the entire experiment.

After more than five months on these diets some of the females were impregnated by stock males and litters were produced. After the young were weaned, the mothers were given the usual resting period of one month, and then attempts were made to remate them. Completely failing this, the animals from both groups were deprived of the vitamin B_1 and B_2 factors and instead for one month were given 2 per cent yeast as a sole source of vitamin B. They were then returned to their respective B_1 and B_2 diets. At the end of this time successful matings with stock males, were secured in five of the seven original mothers from each group. At this time the females had been on the respective diets for eight months (with the exception of the one month on 2 per cent yeast).

Comparison of first and second litters. The birth and lactation reports of these young have been presented in paper II of this series (3). Because of the similarity in all findings up to weaning, first and second litters are there grouped together (with other young born on the same diets). In that paper it was indicated that on such diets, as compared with stock, were obtained 1, fewer young per litter; 2, smaller birth weights, and 3, a high mortality. However, weight gains for the surviving young during the lactation period were the same as for our normal stock animals in the case of diet CXX and not significantly lower for that of CXIX.

Compared with each other, no difference of any significance was obtained between first and second litters in number of young per litter, average birth weights, average weight gains to weaning, or mortality.

Experimental groups using first litter young. After weaning the first litters, the young from each of the original diets were segregated into

groups to investigate the following points: 1, the effect of continuing the offspring on the original diets of their mothers, respectively CXIX and CXX; 2, the effect of withdrawing the factor which had been supplied in the lesser proportion in the original diet, leaving respectively only *a*, 10 per cent B₁, and *b*, 10 per cent B₂; 3, the effect of withdrawing both factors, viz., reduction to a vitamin B free diet; 4 the effect of the substitution of 2 per cent yeast in place of the B₁ and B₂ preparations; 5, the effect of supplying 10 per cent of each of the B₁ and B₂ factors. On the twenty-eighth day of the experiment, two animals from each group (5) above were transferred to ascertain (5') the effects of supplying 20 per cent of each of the B₁ and B₂ factors.

The vitamin B portions of the diets then varied as follows;

<i>Young from CXIX mothers</i>		<i>Young from CXX mothers</i>	
	<i>Number of rats</i>		<i>Number of rats</i>
1. 10 per cent B ₁ 2 per cent B ₂ .	4	1. 5 per cent B ₁ 10 per cent B ₂ .	4
2. 10 per cent B ₁ No B ₂	6	2. No B ₁ 10 per cent B ₂	5
3. No vitamin B.....	5	3. No vitamin B.....	5
4. 2 per cent yeast.....	5	4. 2 per cent yeast.....	4
5. 10 per cent B ₁ 10 per cent B ₂ .	6	5. 10 per cent B ₁ 10 per cent B ₂ .	4
5'. 20 per cent B ₁ 20 per cent B ₂ . (2)		5'. 20 per cent B ₁ 20 per cent B ₂ . (2)	
Total.....	26	Total.....	22

First litter groups. Numbers of males and females were approximately equal and were evenly distributed among the groups. This series was continued for a period of sixty days. The average growth curves are seen in the accompanying chart.

Experimental groups using second litter young. After weaning the second litter young, 16 on the CXIX diet and 15 on the CXX diet were available for this experiment. After consideration of the findings from the first group of experiments, it was decided not to repeat all of those tests. Instead, the second litter young were divided into four groups in the following order (males and females again being evenly distributed):

Group 1. Six animals from CXX mothers, placed on a diet containing 20 per cent B₂ no B₁.

Group 2. Five animals from CXX mothers, placed on a diet containing 15 per cent B₁ 20 per cent B₂.

Group 3. Six animals from CXIX mothers and 5 animals from CXX mothers, placed on a diet containing 20 per cent B₁ 20 per cent B₂.

Group 4. Nine animals from CXIX mothers, placed on a diet containing 50 per cent B₁ 20 per cent B₂.

(Please note again that the vitamin rations are not actual percentages, but indicate amounts based on 10 grams basal food per day per animal as 100 per cent. Thus "50 per cent B₁" means that 1.25 gram of the corn meal concentrate was fed each animal daily.)

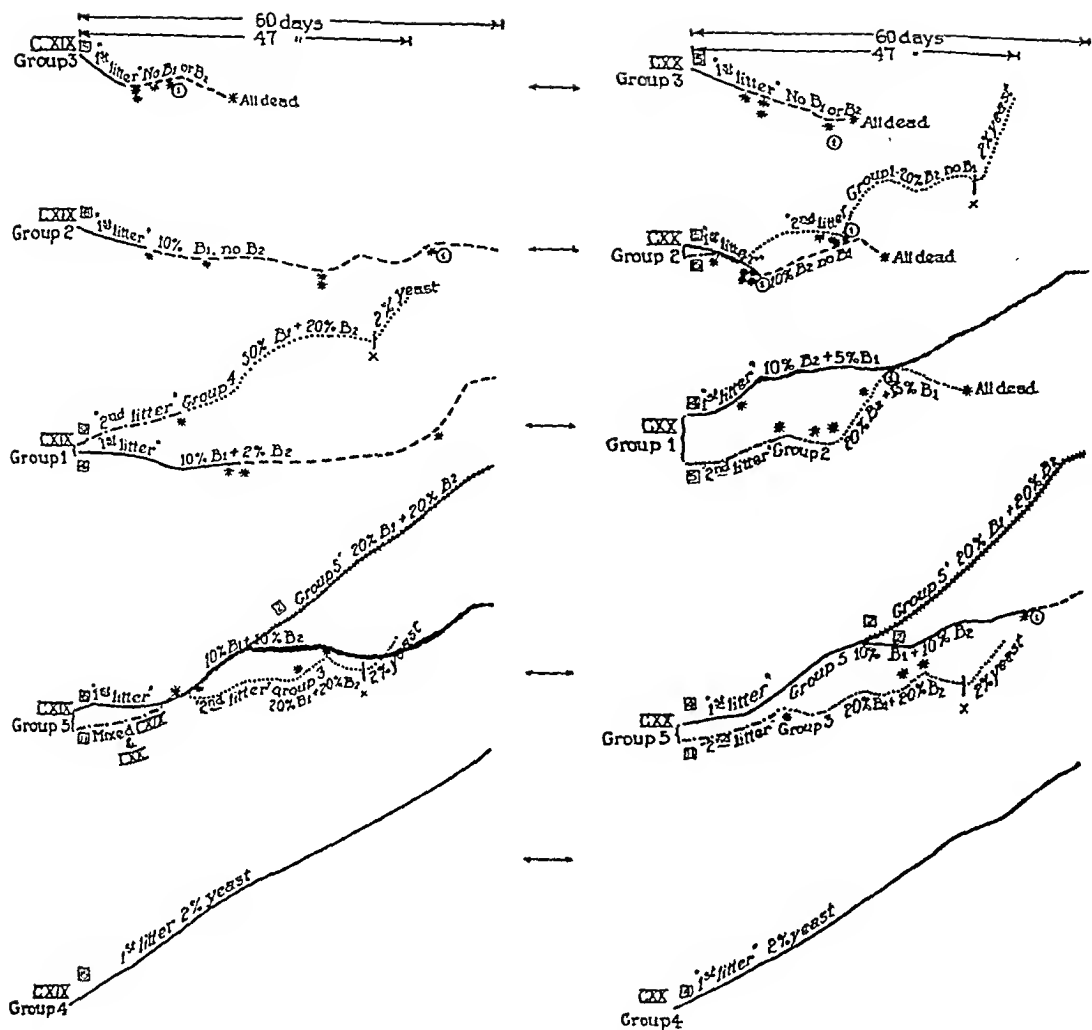
It was hoped in this grouping to enhance such effects as might be associated with predominance of either factor in the mothers' diets. This series was continued for 42 days, after which for 5 days 2 per cent yeast was substituted in place of the B_1 and B_2 factors.

Arrangement of growth curves. The resultant average growth curve for each group is given on the chart, the second litter group accompanying that of the first litter group in which the heredity and diet are most comparable. Thus second litter group 1 is compared with first litter CXX-2, since the young in both cases were from CXX mothers and the diets supplied no B_1 . Second litter group 2 is compared with first litter CXX-1, since again the mothers were from diet CXX and the group diets supplied both B_1 and B_2 factors with the latter in the greater proportion. Second litter group 3 is compared to the 5 and 5' groups from both CXIX and CXX first litter groups, since both maternal diets are represented, and B_1 and B_2 are supplied in equal proportions. Second litter group 4 is compared with first litter CXIX-1, since the maternal diet was CXIX in each case, and also the diet of the young contained both B_1 and B_2 with the B_1 predominating. The last curve may be compared also to CXIX-5', since both the B_1 and B_2 factors were supplied in relatively large amounts.

It will be noted in the following discussions that there is considerable variation in the response of individual animals to certain diet deficiencies. The majority of a diet group may die very early, while a single individual of the same heredity may survive and even show some gain in weight. It was impossible in this experiment to compensate for such a factor of error by the use of large numbers of animals. While it is of importance to consider the animal which acts atypically, we feel that the behavior of the majority of animals in the group should be chosen as the standard for comparison. Apparent gains in weight, due to deaths of smaller animals, should be proportionately discounted on this basis.

Analysis of first litter growth curves with clinical symptoms. Group CXIX-1: Four rats from CXIX mothers continued on the 10 per cent B_1 2 per cent B_2 showed little gain during the 60 day period, the occasional slight rises in the curve usually being associated with the deaths of the smaller animals. One death occurred on each of the twenty-first, twenty-second, and fifty-second days. (Only one animal remained alive throughout the experiment.) On this diet the animals showed an increasingly unkempt condition, the hair becoming ragged, greasy, and yellow. They were generally inactive and walked humped up, on their toes, and with a rolling gait as their condition became worse.

Group CXX-1: Four animals from CXX mothers continued on the 5 per cent B_1 10 per cent B_2 . (On the fifteenth day one of these escaped from the cage and was discarded from the experiment.) They showed a small but steady gain. The only clinical symptoms noted on this diet were an



CODE

- Number of animals (mixed ♀ and ♂) started on group.
- * Death of one animal.
- All animals in group alive (1st litter young).
- After first death (1st litter young).
- - - - - All animals in group alive (2nd litter young).
- After first death (2nd litter young).
- ++++++ 2 animals transferred to 20 per cent B₁ + 20 per cent B₂ on 28th day.
- ⊙ Only one animal remaining.
- × Changed to 2 per cent yeast on 42nd day.

Fig. 1. Growth curves comparing first and second litter young born of mothers that were reared from weaning time on synthetic diets containing varying amounts of B₁ and B₂.

The basal diet consisted of casein 18, dextrin 78, salt mixture 4 and iodine salts with 1 cc. cod liver oil and 4 drops wheat germ oil plus measured amounts of yeast (groups 4) or of extracts of yeast and corn meal for the B₁ and B₂ factors (groups 1, 2 and 5). Groups 4 on 2 per cent yeast show approximately normal growth curves. The other curves indicate lack of growth and subsequent death (groups 2 and 3) or varying degrees of growth (groups 1 and 5) in proportion to the amounts of B₁ and B₂ supplied.

occasional tendency to walk on the toes and a greasiness of the hair which appeared intermittently. There were no deaths.

Group CXIX-2: Six rats from CXIX mothers were continued on 10 per cent B_1 but no B_2 was given. The weight curve shows a small loss with occasional apparent slight gains thereafter as the smaller animals died. Deaths occurred on the eighteenth, the thirty-fourth, and thirty-fifth, and the forty-ninth days. (One animal lived throughout the experiment.) The clinical symptoms developed during the period were greasiness, yellowing, and thinning of hair, nasal hemorrhage, an abnormal rolling gait, and general evidences of inanition.

One rat was transferred to a curative diet of 20 per cent B_1 20 per cent B_2 on the tenth day. His curve thereafter showed a rapid and approximately normal gain to the sixtieth day.

(Note: animals removed from the diets for "cure" are counted as deaths on the growth curves, as experience has shown us that death rapidly follows the appearance of deficiency symptoms unless a curative diet is supplied. Weight curves kept on these cure animals are not given in the chart.)

Group CXX-2: Five rats from CXX mothers were continued on 10 per cent B_2 but no B_1 was given. The weight curve shows a steady drop followed by an apparent rise due to the deaths of the smaller animals. Three deaths occurred on the eighth and ninth days and one on the twenty-sixth day. The clinical symptoms presented were very similar to those in CXIX-2 above except that spastic paralysis developed in two of these rats before death.

One rat was transferred to the curative diet of 20 per cent B_1 20 per cent B_2 on the tenth day. This animal was apparently moribund and had to be fed by pipette for several days. After about a week on the cure diet and although improving gradually from his severe state of inanition, he developed a partial paralysis which lasted for almost a week. The same vitamin treatment was continued and he gradually improved, finally beginning to gain normally after about twenty-five days on the cure. After two weeks of normal weight gain another plateau in his curve appeared.

Group CXIX-3: Five rats from CXIX mothers were fed no vitamin B in any form. The weight curve shows an immediate loss in weight and early deaths. Deaths occurred on the eighth, eleventh, and twentieth days. The clinical symptoms were a general unkempt condition, hunched posture, and rolling gait, inanition, nasal hemorrhage, and in two cases a spastic paralysis.

One rat transferred to 20 per cent B_1 20 per cent B_2 on the tenth day showed a steady although slightly subnormal gain.

Group CXX-3: Five rats from CXX mothers fed no vitamin B in any form showed an immediate loss in weight and early deaths. These occurred on the eighth, tenth, twentieth, and twenty-first days. The

clinical symptoms were the same as for group CXIX-3 above with the two cases of spastic paralysis.

One rat transferred to 20 per cent B_1 20 per cent B_2 on the tenth day showed immediate improvement and better than average normal gain to the end of the experiment.

CXIX-4 and CXX-4: Five rats from CXIX mothers were placed on CXIX-4 and four from CXX mothers on CXX-4. All received 2 per cent desiccated yeast as a sole source of vitamin B, this being the minimum amount usually necessary to produce normal growth after weaning. The growth in both groups was steady, equal, and normal as compared to stock animals of the same age. The rats were in excellent condition throughout, alert, active, and well-groomed. There were no deaths.

Groups CXIX-5 and CXX-5: Six rats from CXIX mothers were placed on CXIX-5 and four from CXX mothers on CXX-5, all receiving 10 per cent B_1 10 per cent B_2 . These animals, although appearing to be in excellent condition and keeping alert and active, gained slowly. On the twenty-eighth day two rats from each group were transferred to CXIX-5' and CXX-5' respectively (*vide infra*). The rats remaining on the 10 per cent B_1 10 per cent B_2 diet continued to gain slowly. (One of the animals from CXIX-5 was accidentally dropped and killed on the eighteenth day.) One of the animals from CXX-5, the smallest rat, showed almost no gain over a long period of time and died on the fifty-first day showing some spasticity but no characteristic paralysis.

Groups CXIX-5' and CXX-5': On the twenty-eighth day two rats from CXIX-5 and two rats from CXX-5 were transferred from the diet containing 10 per cent B_1 10 per cent B_2 to one containing 20 per cent B_1 20 per cent B_2 . They showed an immediate improvement and a normal growth to the end of the experiment (60 days), being comparable in every way to the animals on 2 per cent yeast.

Interpretation of results on first litters: Young from CXIX and from CXX mothers showed no marked variation in response when transferred to the same diets (groups 3, 4, 5, and 5'). On 2 per cent yeast normal weight gains were obtained (groups 4). However, what had been a diet adequate to maintain the mothers (transferred from stock at two to three months) apparently normally for over five months, and which permitted gestation, delivery, and approximately normal lactation weight gains, proved inadequate for normal growth of the young after weaning (groups 1). More than double quantities of our respective B_1 and B_2 preparations were necessary to permit normal weight gains in these young (groups 5').

The most typical clinical symptoms manifested by animals on the deficient diets (groups 1, 2, and 3) were hunched posture, abnormal rolling gait, greasy yellowed hair, and nasal hemorrhage. In some of the rats (6 cases) on B_1 deficient and B free diets (groups CXX-2, CXIX-3, and CXX-3) a terminal spastic paralysis was superimposed.

Analysis of second litter growth curves. 1. Six animals from CXX mothers placed on a diet containing 20 per cent B₂ but no B₁ showed a slight weight gain to the first death on the fourth day (graphed with CXX group 2). Subsequent deaths on the eighteenth and twenty-first days caused an apparent sharp gain due largely to the fact that only the largest animal in the group remained. (His curve showed some variation but practically no rise to the forty-second day when 2 per cent yeast was substituted for the B₂ in the diet. The response was then immediate, and the curve angulated sharply to a better than normal incidence.)

2. Five animals from CXX mothers placed on a diet containing 15 per cent B₁ 20 per cent B₂ showed a slight gain up to the first death on the fifteenth day (graphed with CXX group 1). Subsequent deaths on the eighteenth, twenty-first, and twenty-fifth days caused a sudden apparent increase in weight. As in group 2 this was because only the largest animal remained, but this curve dropped steadily till death on the thirty-eighth day.

3. Six animals from CXIX mothers and five from CXX mothers placed in one group on a diet containing 20 per cent B₁ 20 per cent B₂ showed a small gain with some variations, due largely to deaths on the fifteenth, thirty-second and thirty-fifth days. (Graphed with both CXIX and CXX group 5.) The sudden drop thereafter was abruptly reversed by the substitution of 2 per cent yeast for the vitamin B portion.

4. Nine animals from CXIX mothers placed on a diet containing 50 per cent B₁ 20 per cent B₂ gained steadily, with one death on the fourteenth day, until the thirty-first day when the curve flattened out (graphed with CXIX group 1). After the thirty-fifth day it declined until sharply checked on the forty-second day by the substitution of 2 per cent yeast for the B₁ and B₂ ratios.

Clinical appearance of second litter animals. The appearance of the second litter young in group 1, (no B₁ 20 per cent B₂) and group 2 (15 per cent B₁ 20 per cent B₂) was very similar. These animals were rather scrawny and showed the usual greasy, yellowed fur. Typical paralysis was not seen prior to death, although some spasticity did occur in a few cases. Usually the animals sat hunched up and remained apparently somnolent until they fell on their sides from weakness. In a number of cases red edematous swelling of the feet, particularly of the hind feet, occurred.

On group 3 (20 per cent B₁ 20 per cent B₂) and group 4 (50 per cent B₁ 20 per cent B₂) the appearance was somewhat better, although all of the animals showed some greasiness of fur. Animals dying on these groups did not show typical paralysis but presented rather a picture of inanition. Nasal hemorrhage was almost invariably present in all the animals dying on any of the second litter diets.

Interpretation of results on second litters. On none of these diets supplying large amounts of B₁ or B₂ or both was normal growth obtained. Two per cent yeast, supplied on the forty-second day, elicited better than normal gains in all surviving groups. Spastic paralysis was not manifested in any animal on these diets.

Comparison of first and second litter animals. Approximately double the maternal B₁ and B₂ rations were required for normal growth after weaning in first litter young, but this amount proved quite inadequate for growth after weaning in second litter young. Both first and second litters responded normally to 2 per cent yeast. Clinical manifestations of deficiency in first and second litter young were very similar, with the exception that spastic paralysis occurred in some of the first litter animals, while swollen, red feet were seen in part of the rats from the second litters.

SUMMARY AND DISCUSSION. At the time this investigation was being carried out we were at a loss to explain, on the basis of B₁ or B₂ deficiency, the discrepancy in results obtained. Our B₁ and B₂ products, while admittedly not absolutely pure, still contained such high concentrations of the respective vitamin factors as to produce diverse, distinctive clinical pictures in animals taken from stock diets. Cures were readily brought about by administration of adequate dosages of the appropriate vitamin preparation. The rigidly standardized technique of preparing our B₁ and B₂ products seemed to preclude possible noticeable variations in their potency or nature. Previous investigations had indicated to us that our B₂ product contained traces of B₁ and probably of some other factor which was present in yeast. Our B₁ product we had shown to be practically free from B₂. We had had no indications that another factor might be present.

We had come to consider that 10 per cent as a maximum ration of the B₁ or B₂ preparations permitted optimum development.

In the present study the mothers (transferred from stock at 2 and 3 months of age) appeared to develop normally on 10 per cent B₁ 2 per cent B₂ and 5 per cent B₁ 10 per cent B₂ diets. The first litter young of these mothers born after five months on the synthetic diets, showed approximately normal weight gains to weaning. After weaning, however, when continued on the maternal diets they showed very inadequate growth. Normal growth in these young was possible only when the B₁ and B₂ portions were approximately double those in the maternal diets or when 2 per cent yeast was supplied as a source of vitamin B.

Second litters, born after eight months, could not be obtained until the mothers had been supplied with 2 per cent yeast for one month prior to impregnation. During lactation these second litter young showed approximately normal weight gains. After weaning, when placed on B₁ and B₂ rations which had proved adequate for normal growth of first litter young,

these second litter animals made very inferior progress. However, they responded immediately to 2 per cent yeast.

The clinical symptoms occurring in the young of either first or second litters varied from the characteristic pictures of either B_1 or B_2 deficiency. In the first litters deaths occurred on diets previously considered as adequate, while in the second litters animals died on diets containing relatively very high percentages of the B_1 and B_2 preparations. We found hunched up posture, abnormal rolling gait, greasy yellowed fur, and nasal hemorrhage as a characteristic clinical picture. In six of the first litter animals a terminal spastic paralysis was superimposed on this. Of the second litter young, none showed terminal paralysis, although some spasticity occurred in a few cases. Moribund animals invariably presented the picture described above with the addition in some cases of red edematous swelling of the feet, usually appearing only in the hind feet.

Reader (3) describes the clinical picture of B_4 deficiency as contrasted to polyneuritis resulting from a lack of B_1 . We were surprised to find that her report gives identically (with the exception of nasal hemorrhage) a description of the condition we had found in our animals.

It appears evident then that in this study our results are closely associated with a lack of B_4 . Assuming this to be true, the following explanation of our findings appears logical.

Preliminary work with our B_2 preparation (4) led us to believe that it contained traces of some other vitamin factor present in yeast as well as small quantities of B_1 , since on 10 per cent B_2 we could raise part of our animals to maturity. That this third vitamin factor was probably B_4 was indicated when Reader described a factor which was present in yeast and necessary for the continued growth of the rat. In the present study, the better growth obtained on 50 per cent B_1 20 per cent B_2 than on 20 per cent B_1 20 per cent B_2 seems to indicate that small quantities of B_4 were probably present also in our B_1 preparation.

Storage of B_4 in our stock animals, together with the quantities of B_4 supplied as impurities in our B_1 and B_2 products permitted normal development of mothers of litters used in this experiment. Lesser storage of B_4 in the first litter young was indicated by their inadequate weight gains when continued on maternal diets, and by the appearance of B_4 deficiency clinical symptoms.

Although themselves apparently normal, the mothers had to be "re-stocked" with some B_4 (by giving 2 per cent yeast) before second litter could be obtained. These young showed very much less storage of B_4 since they required very large quantities of our B_1 and B_2 preparations, but the amount of B_4 so supplied was insufficient to produce normal growth in any of the diets used. These young died on much more than adequate B_1 and B_2 rations, showing typical symptoms of B_4 deficiency. Young

from both litters responded immediately and with adequate weight gains when supplied with minimal amounts of yeast.

It would seem then, that our work, through lack of a third vitamin factor found in yeast, closely corroborates that of Reader on vitamin B₄.

CONCLUSIONS

In order to explain our findings we must assume:

1. The presence in yeast of a third vitamin B factor. We identify this with Reader's B₄.
2. That deficiency of this factor produces a characteristic clinical picture distinct from those of B₁ or B₂.
3. That this factor is necessary for continued growth in the rat.
4. That this factor is stored in progressively lesser amounts in *a*, mothers from stock placed on synthetic diets; *b*, first litter young, and *c*, second litter young of these mothers.
5. That more of this factor is required for the production of litters than is necessary for the maintenance of adult females.
6. That this factor is present in mothers' milk in amounts adequate to produce normal weight gains in the young to weaning, even when the maternal diet is relatively deficient in this accessory.

BIBLIOGRAPHY

- (1) MOORE, PLYMATE, ANDREW AND WHITE. *This Journal*, 1932, cii, 566.
- (2) HUNT. *Journ. Biol. Chem.*, 1928, lxxix, 723.
KENNEDY AND PALMER. *Journ. Biol. Chem.*, 1928, lxxvi, 591.
- (3) READER. *Biochem. Journ.*, 1929, xxiii, 689; 1930, xxiv, 77.
- (4) MOORE, PLYMATE, ANDREW AND WHITE. *This Journal*, 1932, cii, 573.

STUDIES IN THE B VITAMINS

IV. A REPORT OF LITTERS OBTAINED ON A DIET IN WHICH FECES WERE SUPPLIED AS A SOLE SOURCE OF VITAMIN B

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The possible importance of the vitamin B content of feces as a factor influencing results obtained with rats on diets low in this food accessory was pointed out by Steenbock, Sell and Nelson (1). They found that growth was doubled when such rats had access to their excreta. McCollum, Simmonds and Becker (2) questioned these findings on the basis of possible errors of method and reported very comparable results on rats with and without access to their feces. Salmon (3) in a carefully conducted series of experiments using fresh, old, and fungus-grown feces corroborated Steenbock's findings. He indicated that even with wire-bottomed cages a certain error was possible, though of probable slight importance, because of the great avidity with which rats on a low-B diet would seize and devour the droppings.

We wished to determine *a*, if feces in the diet as a sole source of vitamin B were adequate to permit growth to maturity of animals born of mothers raised on low vitamin B diets; *b*, if litters could be secured from such animals.

Survival on feces diet. On the test experiment, 10 adult stock males were transferred from the stock diet to one in which 0.1 gram of dried brewer's yeast per animal per day was supplied as a sole source of vitamin B. These animals were kept in screen-bottomed cages, two meshes to the inch, and the droppings were collected daily. These feces, throughout the course of the experiment, formed the sole source of vitamin B in the diet of the group described below.

Experimental group: 25 animals mixed males and females, 2 to 3 months of age, born and weaned by mothers raised on low vitamin B diets¹ were placed in wire-bottomed cages (four meshes to the inch) and supplied our usual basal diet, and cod liver oil, wheat germ oil, and the fresh feces of the group of males mentioned above as a sole source of vitamin B.

Control groups: Our controls were made up of four groups of animals transferred from their original diets to a vitamin B free diet as indicated.

Group 1. Nine adult rats transferred from stock.

Group 2. Eight partly matured rats transferred from stock.

Group 3. Five partly matured rats transferred from diet CXIX.¹

Group 4. Five partly matured rats transferred from diet CXX.¹

All of these animals were kept in wire-bottomed cages and did not have access to their feces.

Summary of time of death on B-free diet

	DEATHS										
	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	9 weeks	10 weeks	11 weeks
Group 1. (9 adults from stock).....							1	2	6	All dead	
Group 2. (8 young from stock)†.....						1	(1*)			2(3*)	1
Group 3. (5 young from CXIX).....		4		1	All dead						
Group 4. (5 young from CXX).....		3		2	All dead						

* Rats showing deficiency symptoms, cured by administration of adequate quantities of yeast. All the animals in this control experiment died as shown except the four that were given yeast.

† Four dead and four cured in this group.

Of the 25 animals on the test experiment (of the same parentage and diet as groups 3 and 4 above) receiving the same diet as the control groups with the exception that feces were added, 19 were still alive at the end of 8 months. At 10 months nine were living. The animals had now reached the age limit (12 months) set by this laboratory in all investigations of pregnancy or lactation, so the experiment was discontinued.

Litters on feces diet. The possibility of procuring litters on this very poor diet was of particular interest to us. The 25 rats on the feces diet described above were used. At first the males and females were segregated and attempts made to mate the females with stock males, placing the pairs together overnight. This being unsuccessful, the males and females on the feces diet were mixed. During several months eleven litters totaling 46 young were obtained (one female produced two litters).

We wish to emphasize that both parents had been raised on the "feces" diet.

For control, 8 stock females were impregnated and transferred immedi-

¹ CXIX and CXX, respectively 10 per cent B₁ + 2 per cent B₂, and 5 per cent B₁ + 10 per cent B₂, described in paper III of this series (4).

ately to a vitamin B-free diet. At the end of gestation 58 young were delivered.

In the accompanying tables (1, 2 and 3) are given the statistical results of our data on stock litters as compared with litters obtained on the feces diet, and the litters from mothers transferred from stock to a vitamin B-free diet when pregnant. The tables compare the number of young per

TABLE 1
Number of young per litter

DIET	NUMBER OF LITTERS	NUMBER OF YOUNG	RANGE IN NUMBER OF YOUNG	MEAN NUMBER OF YOUNG	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	DIFFERENCE OF MEANS	
							RANGE STANDARD DEVIATION	PROBABLE ERROR dif.
Stock.....	189	1,624	2-16	8.59	± 0.13	± 2.59	5.8	
B-free (when pregnant).....	8	58	4-10	7.25	± 0.52	± 2.16	3.2	2.5
Feces.....	11	46	2-9	4.00	± 0.42	± 2.05	3.9	7.7

Rats depending on feces for vitamin B complex content of diet showed decidedly fewer young (4.00) in their litters (feces from animals on low vitamin B diet). Rats impregnated before being placed on a B-free diet were not significantly different from stock in number per litter (7.25 compared with the 8.59 in stock).

TABLE 2
Average weight of young at birth

DIET	NUMBER OF LITTERS	NUMBER OF YOUNG	WEIGHT RANGE	MEAN WEIGHT	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	DIFFERENCE OF MEANS	
							RANGE STANDARD DEVIATION	PROBABLE ERROR dif.
			grams	grams				
Stock.....	164	1,379	3.7-6.5	5.27	± 0.03	± 0.58	5.0	
B-free (when pregnant).....	7	51	3.8-5.0	4.34	± 0.09	± 0.35	3.4	6.6
Feces.....	7	49	3.0-4.7	3.80	± 0.11	± 0.53	3.2	8.8

Mean birth weights are significantly lower than stock (5.27) both in litters born on a B-free diet (4.34) (mothers transferred to diet when pregnant) and litters born on a diet with feces (3.80) as a sole source of vitamin B complex (feces from animals on low vitamin B diet).

litter, the average weight of young at birth, and the mortality during lactation on these diets. The mean number of young per litter born on the feces experiment (4.0) is very much lower than on stock (8.59) while rats transferred to a B-free diet (7.25) when pregnant show no significant difference from stock. Young both on the B-free and the feces diets showed very low birth weights, that on feces being the lower (considerable storage

of vitamin B is indicated in the stock mothers transferred to a B-free diet when pregnant). Mortality of young during lactation was 100 per cent for both experimental groups, except for three young transferred from the B-free to stock mothers during the third week (these developed normally). Most of the deaths occurred during the first week. On the feces diet all died within two days with the exception of five young from one litter which survived to the third and fourth weeks and then died.

TABLE 3
Mortality of young during lactation

DIETS	NUMBER OF LITTERS	NUMBER OF YOUNG	PER CENT OF TOTAL DEATHS DURING LACTATION										TOTAL MORTALITY	
			First week		Second week		Third week		Fourth week					
			Mortality										Number	Per cent
			Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent				
Stock.....	189	1,624	66	82.5	4	5.0	7	8.8	3	3.8	80	4.98		
B-free (when pregnant).....	8	58	45	77.6	7	12.1	1 (3*)	6.9	2	3.4	55 (3*)	100.00		
Feces.....	11	46	41	88.0			2	4.3	3	6.5	46	100.00		

* Three young from the B-free diet were transferred to stock for cures during the third week. These were in bad condition and would have died had they been left with their mothers. They are therefore counted as mortalities in this table.

This table demonstrates that 4.98 per cent of 1624 "stock" young died during lactation whereas 100 per cent of the other two groups died. Also 77.6 per cent or more of the mortality before weaning occurred during the first week.

SUMMARY

1. The feces from ten rats on a diet supplying 1 per cent yeast was found sufficient to keep the majority of 25 young rats alive for over eight months when used as their sole source of vitamin B.

2. Eleven litters, totaling 46 young, were obtained over a period of several months from parents raised on a diet which supplied feces as a sole source of vitamin B.

3. Statistical results of data on litters from *a*, stock; *b*, the feces diet, and *c*, a B-free diet (females transferred from stock when pregnant) are given. The litters on the feces diet had decidedly fewer young and a very low birth weight. On the B-free diet the number of young per litter was not significantly different from that in stock, but the birth weights were much lower.

4. The mortality of the young during the lactation period was 100 per cent in both the "feces" and the B-free groups.

5. In all groups over 77 per cent of the mortality before weaning occurred during the first week.

BIBLIOGRAPHY

- (1) STEENBOCK, SELL AND NELSON. Journ. Biol. Chem., 1923, lv, 397.
- (2) MCCOLLUM, SIMMONDS AND BECKER. Journ. Biol. Chem., 1925, lxxiii, 547.
- (3) SALMON, W. D. Journ. Biol. Chem., 1925, lxxv, 457.
- (4) MOORE, PLYMATE AND ANDREW. This Journal, 1932, cii, 581.

STUDIES IN THE B VITAMINS

V. A STUDY OF MYELIN DEGENERATION IN THE PERIPHERAL NERVES OF RATS AS ASSOCIATED WITH LOW VITAMIN B CONTENT OF DIET

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A very extensive literature exists on the subject of pathological changes in the nervous system of animals suffering from vitamin B deficiency. No attempt is made here to go into this literature in detail. The studies of Jackson (1) and of Stern and Findlay (2) give historical reviews of the most important work done up to 1929. These indicate the diversity of findings and opinions among different workers in this field. Myelin degeneration in the peripheral nerves of young rats on low vitamin B diets has been reported by this laboratory (Moore, Brodie, and Hope, 3).

Since our work for the past several years has been concerned with the study of various phases of vitamin B deficiency, using relatively large numbers of animals, the time was considered propitious to make an extended investigation of myelin degeneration of the peripheral nerves of rats as associated with low vitamin B content of the diet. The selection of typical animals from our various diet groups enabled us to obtain a generalized and carefully controlled picture.

The study here presented was made on a total of 1,714 slide preparations obtained from 471 rats chosen from 19 of our diet groups (4).

We hoped from this series to determine whether myelin degeneration occurred with sufficient regularity—if at all—to be considered as a valid criterion of vitamin B deficiency in rats.

In the previous work of our own laboratory the findings had been varied, at times no changes being noted, and at others what appeared to be Wallerian degeneration being found. We hoped to discover, if possible, the cause of this discrepancy. We wished to determine whether true Wallerian degeneration could be found in animals of any age on any of our diets. The possibility of apparent myelin degeneration occurring due to such factors as post-mortem changes or mechanical injury occasioned by methods of handling the nerves was investigated. (The latter was suggested by McCarrison in his criticism of the degeneration test (5).) Also the rate

and extent of myelination of the nerves in young animals on stock and experimental diets was compared.

It was recognized that a more rapid post-mortem degeneration or a retarded myelination in the nerves of rats on poor vitamin B diets might be almost as significant as a true degeneration.

Animals used: Typical animals of varying ages were taken from stock and from 18 experimental diets. For the sake of brevity in reporting these, individual diets are not enumerated, but those which were similar have been grouped under appropriate general headings. Four hundred and seventy-one rats were employed of which 83 died or were killed in extreme stages of paralysis or inanition. A total of fifty young taken from the different diets were used in the myelination study.

Killing of animals: Part of the rats used had died from accidental mechanical injury. A few were killed by cutting the jugular vein or by etherizing lightly and then cutting the jugular vein. Most of our animals, however, were etherized. In some of these the nerves were removed while the animals were still living, and in some after death. Other animals died of inanition or paralysis produced by diets deficient or lacking in B₁, B₂, or the B complex. No variations could be demonstrated in the nerve preparations as a result of these different methods of killing the animals.

Nerves: In all cases, except those indicated, nerves were taken immediately or very soon after death. Only peripheral nerves were used, the most common being the sciatics, the phrenics, the vagi, and the brachial plexuses. The larger branches were ordinarily studied, no investigation being made of the nerve endings. (For a consideration of nerve endings in rats on a low vitamin B diet see Woollard, 6.)

Technique of nerve preparations: After some preliminary work with the Marchi and the sodium iodate techniques, osmic acid stain for peripheral nerves was employed. Three of the common variations of fixation were used: 1, fixation and staining with osmic acid solution; 2, short fixation (6 to 12 hours) in formalin with subsequent staining with osmic acid solution; 3, long fixation in formalin (over 12 hours), staining with osmic acid solution. All nerves were preserved in glycerin and teased and mounted in glycerin jelly. Teased nerves were used exclusively in this study; no sections were made.

The Marchi method, although eminently satisfactory, proved impractical for the extent of the work with the limited facilities at our command. The sodium iodate method we found not to be dependable in our hands. The osmic acid stains were simple and reliable, the osmic fixation being somewhat preferable as it was free from the artefacts of the formalin fixation.

Slides and examination: An average of four slide preparations was made from each animal, although there was considerable variation; 1,714 slide preparations were made. These were examined individually by two

workers and separate reports made before notes were compared. The results agreed on all essential points.

Control Wallerian degeneration: Wallerian degeneration controls were prepared using three young adult stock animals. Each rat was lightly etherized, a small incision made in the left leg, and the sciatic nerve trunk cut. At five and ten days the animals were again etherized and portions of the nerve peripheral to the point of sectioning were removed. On the tenth day portions from the whole right sciatics were also taken for comparison (these were normal).

Nerve preparations made from the degenerated nerves were used as a standard of true Wallerian degeneration.

Diet groups investigated: Typical animals chosen from the various diet groups are best listed in the following table.

DIET GROUP	AGE (MONTH)			TOTAL ANIMALS	RATS SHOWING	
	1 to 4	4 to 12	Over 12		Paralysis	Inanition
Stock.....	57	52	29	138		
10 per cent yeast.....	25	36	3	64		
2 per cent yeast.....	12	15		27		
B ₁ +B ₂ diets.....	58	31		89	18	8
B ₁ diets.....	24	3		27	2	3
B ₂ diets.....	17	12		29	12	2
B-free.....	21	26		47	4	17

(Fifty young, of which 12 were paralyzed, used in the myelination studies are not included in this table.)

In none of the nerve preparations made from these animals was any condition resembling Wallerian degeneration apparent. We did find in some cases, however, a foamy appearance similar to that described by Schaumann (7) in nerves of rats on a generally low vitamin diet. Also some swelling of the myelin sheaths was found, as in the work of Hofmeister on rats suffering from vitamin B deficiency (8). However, both of these conditions appeared as frequently in our preparations from animals on stock and adequate vitamin B diets as in those deficient in vitamin B. We cannot therefore postulate a causal relationship between these myelin changes and an inadequate supply of vitamin B in the diet. No consistent changes were found to characterize nerves of animals on low vitamin B diets.

Study of post-mortem changes in nerves: Sixteen adult rats, 10 normal from stock, 3 normal from our 10 per cent yeast diet, and 3 suffering from advanced malnutrition on a B-free diet were used. The animals were killed with ether. Small control sections were taken from one set of nerves immediately and the bodies were kept at room temperature. At

the intervals indicated below, later sections were taken from cut nerves (both distal and proximal ends) and from the uncut nerves. Sciatics, brachial plexuses, vagi, and phrenic were used.

A list of the slides prepared follows.

Hours.....	Immediate	2	5	6	8	12	18	24	30	36
Slides.....	16	73	90	71	36	16	16	8	8	10

Results: No clear degeneration was discernible until twelve hours or more had elapsed. From that time it became more and more evident, so that by twenty-four hours it was very marked. No difference could be demonstrated between cut or uncut nerves. Nor was there variation in the time of appearance or extent of degeneration for the different diet groups.

Mechanical injury of nerves: For this study adult animals were taken from all of the synthetic diets and a complete age range (after weaning) of stock rats. In each case as long a portion of the nerve as possible was carefully removed from the animal. One or two sections were always saved as controls, one portion was dried until quite stiff and brittle, one crushed with forceps, and one stretched vigorously. When teasing the nerves, adjacent portions of one or more control segments were reserved for heating. In approximately half of the cases the nerves were examined in glycerin jelly before heating, then heated somewhat and again examined, and the process repeated. This produced a perfect control, making it possible to see changes in individual fibers.

A list of the preparations made follows.

	CONTROL	DRIED	STRETCHED	CRUSHED	HEATED
Stock.....	80	14	42	41	7
Experimental.....	123	25	101	67	17

Results: The nerves heated slightly showed no variation from the controls. If heating was continued they shrank and became twisted. Due to shrinkage they became darker. If heating was extensive they twisted into very compact bunches and became black or almost so. As long as the nerve structure could be determined it was like the control. In no case could a normal nerve after heating have been mistaken for a degenerated one.

Nerves if dried slightly showed no consistent variation from the controls.

With extensive drying they became very compact and were teased with difficulty. The stain was dark or black, the nerves brittle, breaking as readily across as between the fibers. The microscopic appearance of these nerves in no way resembled myelin degeneration.

Crushing the nerves tended to break the myelin and in some cases caused extrusions of myelin through the sheath. This was widespread or hardly noticeable depending on the severity of treatment. However, the myelin damage caused by crushing was sharp and irregular, showing none of the characteristic globular particles found in degeneration. The ordinary observer would not mistake one for the other.

After stretching, if not held taut during fixation, the nerves twisted extensively. Pulling tended to break the nerves or the myelin within the nerves, but there was no appearance simulating degeneration due to it.

It was possible to differentiate grossly between the unteased nerves by their appearance and feeling. Dried nerves were stiff, relatively thin, and rather brittle. Crushed and stretched nerves were soft, pliable, relatively large, and broke into their fibers readily. The controls (untreated nerves) ranged between the two.

These differences persisted for at least fourteen months when the nerves were preserved in glycerin after staining.

No variation in appearance could be demonstrated between nerves from the different diet groups.

We conclude that mechanical injury due to heating, crushing, stretching or drying of the peripheral nerves does not produce changes in them which could be interpreted as myelin degeneration.

Myelination studies: Our interest in this subject depended on its influence on qualitative and practical histological tests as concerned: *a.* The possible mistaking of myelination for myelin degeneration. *b.* The possible retardation of myelination in rats on diets deficient in vitamin B. Should the second possibility prove apparent a thorough quantitative study would, of course, be indicated in view of its interesting theoretical possibilities. Our investigation of myelination of the peripheral nerves of the rat was in no sense undertaken as a re-checking of the quantitative work done in this field at the end of the last century (8). (Our technique could show only qualitative differences.)

The following preparations were made of the nerves of young from stock litters and young killed and dying from litters whose mothers were on low vitamin B diets. Fifty young were used, of which, on the low B diets, 12 showed typical paralysis. The young obtained at two weeks gestation were taken alive by Caesarean section on normal stock mothers.

Nerve preparations studied

	GESTATION TWO WEEKS	BIRTH	WEEKS TO WEANING			
			One	Two	Three	Four
Stock.....	8	8	11	42	59	22
Low B diets.....			8	12	40	22

In premature young taken by Caesarean section at two weeks' gestation or in young at birth no myelination in the peripheral nerves could be demonstrated. As had been shown by earlier workers (8), myelination was found to be slight at one week, to advance steadily during the next two, and to be practically completed by the fourth. The forming myelin globules as seen in the teased sections obviously resembled myelin degeneration and could readily be mistaken for that process. It seems probable that without careful controls the myelination process in young rats may be misinterpreted as myelin degeneration.

The nerve preparations from paralyzed young were not distinguishable from the controls. By our methods no indications of retarded myelination associated with a deficiency of vitamin B could be shown even in the case of paralyzed young.

SUMMARY AND CONCLUSIONS

1. This study was made on 471 rats, chosen as typical, from our stock and 18 of our synthetic vitamin B diets. Of these, 83 animals died or were killed in late stages of inanition or paralysis due to vitamin B deficiency. Only peripheral nerves were studied.

2. A total of 1,714 slide preparations was made, using the osmic acid staining and teased nerve techniques exclusively.

3. No true Wallerian degeneration was seen in any of the nerve preparations other than in the Wallerian degeneration controls and late post-mortem preparations.

4. A foamy appearance of the myelin and swelling of the myelin sheath was seen as frequently in our controls as in our low vitamin B diets. No causal relationship between these changes and an inadequate supply of vitamin B in the diet could be demonstrated.

5. A study of post-mortem changes in nerves left in the body at room temperature showed that no myelin degeneration appeared until 12 hours or more had elapsed.

6. No acceleration of post-mortem degeneration was demonstrated in nerves of rats on deficient vitamin B diets.

7. Mechanical injury of nerves occasioned by heating, drying, crushing, or stretching did not produce myelin changes simulating Wallerian degeneration.

8. The myelination process might readily be misinterpreted as myelin degeneration in young rats.

9. Myelination was not found to be retarded in young rats on low vitamin B diets, by the methods used.

10. No consistent changes in the nerves were found by our methods to characterize vitamin B deficiency in rats.

11. We do not consider myelin degeneration in the peripheral nerves of rats a valid criterion of vitamin B deficiency.

BIBLIOGRAPHY

- (1) MOORE, BRODIE AND HOPE. *This Journal*, 1927, lxxxii, 350.
BRODIE. *This Journal*, 1929, lxxxix, 340.
- (2) JACKSON. *Arch. Path.*, 1929, viii, 81, 273.
- (3) STERN AND FINDLAY. *Journ. Path. and Bact.*, 1929, xxxii, 63.
- (4) MOORE, PLYMATE, ANDREW AND WHITE. *This Journal*, 1932, cii, 566.
MOORE, PLYMATE, ANDREW, AND WHITE. *Ibid.*, 1932, cii, 573.
MOORE, PLYMATE AND ANDREW. *Ibid.*, 1932, cii, 581.
MOORE, PLYMATE AND WHITE. *Ibid.*, 1932, cii, 593.
MOORE AND PLYMATE. *Ibid.*, 1932, cii, 605.
- (5) McCARRISON. *Studies in deficiency disease*. Oxford Medical Publications, London, 1921.
- (6) WOOLLARD. *Journ. Anat.*, 1927, xli, 283.
- (7) SCHAUMANN. *Beihfte z. Schiffis-u. Tropenhyg*, 1910, xiv, 325; 1912, xvi, 137.
- (8) HOFMEISTER. *Biochem. Zeitschr.*, 1922, cxxviii, 540.

STUDIES IN THE B VITAMINS

VI. FURTHER CONSIDERATION OF PYLORIC OBSTRUCTION IN RATS

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For several years the Nutritional Research Laboratory of the University of Oregon Medical School has been studying problems connected with the effect on the young of inadequate maternal diets during pregnancy and lactation, especially the effect of a deficiency of the vitamin B complex or of B₁ or B₂. From 1925 to 1927 the effects on litters of 2 per cent yeast as the source of vitamin B in pregnant and nursing rats was investigated. This diet was found to be insufficient for normal production and growth of litters in our animals, being associated with hemorrhages of the young and the mother, lowered birth weights, lower weight gains, high mortality, and some polyneuritic paralysis, especially in second generations on this diet (1).

These findings, although indicating a greater need for the B complex in our series of animals than some other laboratories had reported (2), were in line with recognized results of a vitamin B deficiency and were in part corroborated by other workers (3). However, we were surprised to find the appearance of a condition simulating pyloric stenosis in ten of our young on this diet (1.2 per cent of the young in the first generation, 22 per cent of the young in the second generation, 87.5 per cent of all cases being males). This pyloric obstruction was found in none of our normal diets nor in any of our higher percentage yeast diets carried on at the same time. Treatment and subsequent history of the rats developing this condition indicated that there might be a definite relation between it and the food deficiency.

As the personnel of the laboratory was to be changed at the time, it was deemed advisable to report the findings, although the investigation was clearly in its preliminary stages.

In the first paper (4), an experimental study by Brodie, the pyloric obstruction found associated with low vitamin B intake in our rats was discussed in detail. In the second (5), a clinical study by Moore, Brodie, Dennis and Hope, the history and literature of congenital pyloric obstruc-

tion was reviewed. In both papers the possibility of interpretation of the condition as due to a dietary lack of vitamin B, possibly associated with an hereditary tendency, was pointed out. It was obvious that the results were too incomplete to warrant the drawing of definite conclusions; but the indications were clearly worth calling to the attention of other investigators in the field, particularly to clinical workers who had available cases which they could examine and treat with a view to verifying or refuting this vitamin B deficiency hypothesis.

Congenital hypertrophic pyloric stenosis has long been known to be common in particular families, some workers believing it to be actually hereditary (6). In our papers presenting the hypothesis of vitamin B deficiency as the cause of pyloric stenosis it was pointed out that this heredity might be apparent or real, i.e., due to family food habits or to a distinct hereditary need of a high B intake (5). Pathology in vitamin B deficiencies has been found to be so varied and extensive that eventually it may be shown that too low an intake tends to precipitate any of several conditions where there is a predisposition of certain parts of the body to weakness.

Soon after the work above had been done the personnel of the laboratory was changed, and numerous alterations of methods and policy were introduced. Primary among these was the method of administering the vitamin portion of the diet. Previously, a 2 per cent yeast diet had consisted of 2 per cent of the yeast being mixed grossly with the basal food. In this way each animal received a quantity of yeast directly proportional to the amount of basal food he consumed, thus causing a variation from day to day for an individual animal, and between different animals of the group. In order to limit definitely the amount of vitamin B being utilized, a preliminary study to ascertain the approximate average amount of food consumed by each animal was carried out. On this basis it was determined that ten grams of food per animal per day was a generous allowance, and our vitamin portions were calculated on this amount. Thus, an animal receiving 2 per cent yeast as a sole source of vitamin B was now given an accurately measured amount of a yeast suspension in tap water equal to 0.2 gram of yeast daily. Small dishes were obtained, and the entire daily vitamin portion (including cod liver oil (A and D), wheat germ oil (E), and the various measured amounts of yeast (B complex) or of our B₁ or B₂ concentrates depending upon the diet) was accurately measured and served to each animal before the basal portion was placed in the cage (7). In this way we were able to control accurately the vitamin intake of each animal independently of the amount of basal food consumed. There was no attempt made to control the latter factor in any of our experiments, adequate amounts being kept in the cages at all times.

Following the introduction of this method of feeding, three important changes in the clinical results obtained in the animals on our 2 per cent

yeast diet (now 0.2 gm. daily per animal) were noted: 1, almost total disappearance of excessive hemorrhage in the newborn and in the mother at parturition; 2, disappearance of cases simulating pyloric stenosis; and 3, decided reduction in the percentage of cases of typical spastic paralysis, with more of the animals dying of inanition with little or no hypertonicity. These changes we are inclined to ascribe to the fact that the animals now received consistently accurately measured amounts of vitamin B complex and that this amount in all probability was in excess of that consumed by the animals in the earlier experiments.

One of the new experiments instituted at this time was carried out in an attempt to analyze the separate rôles played by the B₁ (F or antineuritic) factor and that of the B₂ (growth G or P-P) factor of the vitamin B complex. The facilities of the laboratory did not permit nor did the nature of our work require absolute purity of our preparations, as we were primarily interested in securing such products as would permit of maintenance of our animals to maturity and if possible pregnancy and lactation of the young to weaning.

Considerable preliminary standardization of the methods of securing and the testing of the potency of our preparations resulted in the adoption of the methods described in paper III of this series (8). In this paper it was pointed out that our B₁ preparation was apparently relatively pure, but that our B₂ preparation (autoclaved yeast) probably contained small amounts of B₁ and also of some other factor or factors then unknown.

On one of our diets, consisting of 1 gram per animal per day of autoclaved yeast (B₂) without the addition of any B₁ concentrate or any other source of vitamin B, one litter was obtained. The mother had been on the following diet for almost nine months previous to impregnation: McCollum's basal mixture 95 per cent, McCollum's salt mixture 4 per cent, cod liver oil, and 1 gram autoclaved yeast daily (no vitamin E was supplied).

- This female had grown normally and appeared healthy. Her hymen did not open until she was approximately six months old and her oestrous cycles were very irregular. After eight months she was impregnated (by a stock male) and had a normal pregnancy. A detailed protocol on her delivery is given below.

Protocol of delivery of female 855, mated to male 1030, September 1, 1928; delivery September 24, 10:15 a.m. (dictated at the time the young were being cared for).

One male young was delivered 10:15 a.m., out of the nest, covered with sawdust, and apparently moribund, gasping for breath every eight to ten seconds without normal breathing reflexes. It was washed with warm water and artificial respiration was administered with the thumb and forefinger at the rate of about seventy per minute for approximately fifteen minutes. Gasping increased progressively until labored inspiratory movements of the diaphragm were begun at about ten minutes; these became progressively more regular until breathing was fairly normal at the end of twenty minutes. The animal was pale and edematous, but this gave way to a normal pink appearance after breathing began.

The cage was again examined (about 10:35 a.m.). Four more young were discovered, breathing normally but covered with sawdust and very cold. These were washed in warm water and warmed in the hand for a few minutes after which they appeared normal.

The cage was again examined, and one female young was found covered with mucus and sawdust, apparently dead. The mother seemed in the act of devouring it, and to prevent her doing so her jaws had to be opened forcibly. The young rat was washed in warm water, mucus cleared from nose and mouth, and artificial respiration administered. Twelve minutes sufficed to elicit the first diaphragmatic efforts, and in fifteen minutes the animal was breathing alone with movements slow and labored but regular.

One more young was found covered with sawdust but breathing normally. No other young were palpable in the mother at 11:25 a.m. The litter contained 4 males, 3 females, no runts, all apparently normal after this treatment. Weight of 7 young, 36 grams. (Average, 5.14 gm., compared with 5.27 gm., stock animals, falls in lower normal range.)

Average litter weight gains during lactation were: one week, 5 grams; two weeks, 12.3 grams; three weeks (5 rats), 13.5 grams; four weeks (1 rat), 17.0 grams. When compared with stock weight gain averages (one week, 6.20; two weeks, 13.63; three weeks, 22.16; four weeks, 31.72) the first two weeks fall in the lower normal range, the last two are definitely subnormal. The mother gained slightly but steadily throughout lactation, showing no ill effects.

The young appeared in excellent condition until the nineteenth day, when it was noticed that they were less lively than before. However, no clinical symptoms of polyneuritis were present until the twenty-first day, when two of the males showed paralysis of the hind legs. One of these was killed and preserved as a control. There was no gastric dilatation. It was attempted to feed the other, a male, an aqueous solution of the antineuritic concentrate (B_1). The rat could not take it and subsequently died with all clinical symptoms of polyneuritis. Autopsy showed the stomach to be greatly distended and the intestines empty except for gas bubbles, but no hypertrophy of the pylorus could be demonstrated.

On the twenty-second day three more young, one female and two males, were badly paralyzed and showed rather extensive blood clots about the nose. The mother also showed this condition. The paralysis of the young in this case seemed to affect the forepaws, which were doubled up. (Moving pictures were made at this time and repeatedly thereafter to the time of the complete cure of the one remaining animal.) In attempts to move about, the young rats walked on the dorsum of the paw almost as frequently as on the palmar surface. The hind legs were affected but were not dragged out posteriorly. The young were unable to balance themselves on their feet. A rubbing of the nose indicative of pruritis was very noticeable. The young were in such bad condition by the afternoon that their lives were despaired of. One of them, the female, was killed as a control

(it showed no gastric dilatation) and the other two, males, were treated as described in the following detailed report, taken from notes made at the time of each observation.

First day. At 3:30 p.m. the animals were too weak to take any food or treatment by mouth. They were apparently moribund, lying apathetically on one side and kicking feebly only when touched. The hair of the top of the head of one was clipped off as a distinguishing mark. This animal was treated with an aqueous filtrate of our B₁ concentrate, 0.1 cc. being injected intraperitoneally and 4 drops being forced down the throat. The other animal was treated with a filtrate of a 20 per cent yeast suspension (macerated for one hour), 0.1 cc. being injected intraperitoneally and 4 drops being forced down its throat. Fifteen minutes later like dosages of the respective filtrates were given. The rats made no attempts to move but lay quietly in a cotton nest near the radiator.

Four hours later the procedure was repeated, 0.2 cc. of each filtrate being used intraperitoneally. The rats appeared somewhat improved by this time and licked rather eagerly at the pipette with which they were fed, each of them consuming about 0.5 cc. An attempt was made to feed them a little of the basal diet made very dilute with water, but only a drop or two was taken.

Second day, 9½ hours after initial treatment. At 1:00 a.m. they were again injected with 0.1 cc. of the respective filtrates, given about 1.5 cc. by mouth and about 10 drops of dilute basal food. They appeared still better, although it was very uncertain whether they would live.

At 4 a.m. they were so much improved that the injection was not given. Each took about 0.5 to 0.75 cc. of its filtrate and a considerable quantity of the basal food. They both ate avidly, although the paralysis was still evidenced by their inability to direct the movements of the head—they would lunge awkwardly at the source of food, licking rapidly the while, then jerk back with a semi-palsied movement. When placed on the floor they were able to maintain their balance much better than before and assayed a few steps.

At 7 a.m. the animals were again fed approximately 0.75 cc. of filtrates and a like amount of basal food. They were much improved and attempted to move about with much better success than previously. By 9:30 a.m., their next feeding, they were improving so rapidly that it was noticeable from hour to hour.

At 12:00 noon they were placed with their mother and left there for the afternoon. It was not determined whether they nursed, but they sought the basal food dish and ate eagerly. At 5:30 p.m., in addition to the usual feeding, the rat receiving vitamin B₁ filtrate was also given a small portion of autoclaved yeast (B₂) which was the mother's diet. Feedings were repeated during the night at 7:30 p.m., 10:00 p.m. and 4:00 a.m. Improvement was continuous and the animals were by this time considered out of danger.

Third day. At 8:45 a.m. each rat was placed in a separate cage and given a dish of basal food, 4 drops cod liver oil, the appropriate filtrate, and some autoclaved yeast for the animal receiving the antineuritic vitamin. Both ate well, the one receiving yeast filtrate with great avidity.

At 12:00 noon and at 1:00 p.m. both animals were apparently in excellent condition, although it was noted that the abdomen of the one receiving yeast filtrate was somewhat distended.

At 2:00 p.m. the rats were again examined. The sun by this time had reached the shelf on which the cages had been placed, and both rats were very warm and panting. (Heat stroke?) They were moved to a cooler place, but the one on yeast filtrate lay

on its side and appeared very ill. A few drops of water were given. It licked these but did not recover further. The body was then immersed in a tepid bath, and it was noticed that the abdomen was immoderately distended. The ruffled appearance of the fur had masked the condition previously. A luer syringe was sterilized and a small needle plunged directly through the abdominal and adjacent stomach walls. Through this was aspirated a small quantity of viscous fluid, which proved, on microscopical examination, to be identical with the food mixture given though mixed with mucus. By this time the animal was gasping painfully and seemed to be dying. A small incision through the abdominal and stomach walls was made and the gastric contents drained. This material was thick and mixed with mucus but still fluid. The animal was too severely affected for its life to be saved. Immediate autopsy showed the stomach walls to be thinned out and stretched to an abnormal degree, but the intestines were empty except for gas bubbles and some hard feces in the lower end of the colon and in the rectum. The other animal was in very good condition.

Fourth day. By the next day the remaining animal showed recovery without any apparent complications. It ran about almost normally, had practically cleaned the accumulated food from its coat, and evidenced the usual inquisitiveness of a young rat of this age (twenty-five days). It had not been returned to its mother, but was kept on the food mixture of basal food, autoclaved yeast, cod liver oil, and antineuritic concentrate.

Fifth day. The following day the animal had completely recovered as far as could be determined. It was very lively. Its coat was clean, soft, and fluffy. Except that it was somewhat smaller than normal it showed no differences from our stock rats. At this time it was decided to deprive it again of all B_1 , its food now consisting only of basal mixture, cod liver oil, and autoclaved yeast. After three days on a B_1 free diet the rat demonstrated the hypersensitiveness and the gait usual in animals approaching paralysis. Also the abdomen was slightly distended.

By the fourth morning on the B_1 free diet, the little animal was definitely ill. There were hard, sticky feces adherent to the anus. A small amount of warm saline was given as an enema with some slight effect. The abdomen was definitely distended, bulging abnormally on both sides in the sub-costal region, and the rat ran about in evident pain.

By 10:30 a.m. the symptoms had become progressively worse. The rat moved about very quickly, but its movements were not well-controlled. It would not eat or drink. There was embarrassment of respiration. The abdomen was enormously distended. To all appearances the animal would have died within a few hours if not treated.

It was therefore lightly etherized. A small slit was made in the skin over the fundal region of the stomach, the stomach was compressed from the right side, and a small hypodermic needle plunged through the abdominal and stomach walls directly into the fundal portion of the stomach cavity. Attempts to aspirate were futile because of the viscosity of the stomach contents, so a syringe containing antineuritic filtrate was attached and 0.3 cc. injected directly into the stomach. This quantity was not sufficient to have diluted the stomach contents to any appreciable extent. On coming out of the anaesthesia the rat appeared to be no worse than before. At 11:15 a.m. it remained in about the same condition. It had survived the operative treatment very well and showed no ill effects.

By 12:00 noon the abdomen showed a decrease in the distention, and the rat was breathing very much easier. By 2:00 p.m. the distention had subsided to practically normal. The animal did not move about in the same agonized manner, but it ap-

peared weak. When offered the antineuritic filtrate it grasped the pipette tightly with both forepaws and drank eagerly all that it was allowed, 0.5 cc. At 6:00 p.m. another feeding of 0.5 cc. of the antineuritic filtrate and a small amount of very dilute basal food was given. The rat seemed almost normal again.

By the next morning it was apparently entirely normal, and one would never have guessed it to be the same animal treated the day previously. It was again given a dish of dilute basal food, autoclaved yeast, antineuritic concentrate and cod liver oil, of which it ate avidly. Two days after treatment it was lively and well.

One other female in the litter died of paralysis on the twenty-fourth day of lactation. There was no gastric dilatation. The one remaining female of the litter although showing some paralysis at the end of the third week recovered without a change of diet and was weaned.

Although repeated attempts were made over a period of fourteen months, using 41 females, to secure young on this diet, only three litters were obtained. The first of these is reported in detail above. In the case of the other two the deliveries occurred during the night and the young did not live. We were therefore unable to duplicate the findings here reported.

We do not wish to draw any conclusions from this one litter, but we think it worthy of consideration in the light of the earlier finding in this laboratory of cases simulating pyloric obstruction in the young born on a diet containing 2 per cent yeast as a sole source of vitamin B. We feel that in the experiments earlier reported from this laboratory, the females on the 2 per cent yeast diet may have received smaller amounts of yeast than did the later animals which received measured 0.2 gram portions of yeast daily.

It is of interest to note, in comparing this with the other cases of apparent pyloric stenosis reported in this laboratory, that yeast has been shown to be relatively lower in the B₁ than in the B₂ factor (9). The minimal amounts of yeast consumed may then have supplied B₁ in such small quantities as to have influenced the production of the pyloric obstruction. This might also account for the occurrence of gastric distention in the case reported above in an animal cured of paralysis by administration of a whole yeast filtrate.

SUMMARY

1. Earlier papers from this laboratory reported 10 cases (87.5 per cent males) of pyloric obstruction in litters born on our diet containing 2 per cent yeast as the sole source of the vitamin B complex. Other clinical findings on the same diet had been hemorrhages of the young and the mother and a high percentage of deaths with terminal paralysis.

2. A change in methods of vitamin feeding was introduced, 0.2 gram of yeast being fed each animal daily in place of the former 2 per cent yeast mixed with the basal food. Subsequently, excessive hemorrhages were

not found, fewer young died of polyneuritis, and no cases of pyloric obstruction appeared.

3. In a series of experiments extending over a period of 14 months and utilizing 41 females raised on a diet in which 1 gram of autoclaved yeast (B_2) was supplied daily as a sole source of vitamin B, only one viable litter was obtained. This delivery was abnormal.

4. At approximately 3 weeks of age one of the four males in this litter of 7 died showing a condition of gastric dilatation resembling pyloric obstruction accompanying typical polyneuritis.

5. One paralysed male killed as a control showed no gastric dilatation.

6. Complete recovery from spastic paralysis was brought about in one male by early intraperitoneal injections and later oral administration of small quantities of a filtrate of yeast. Approximately 34 hours after the initial treatment, and only a few hours after apparent complete cessation of the paralytic symptoms, this animal rather suddenly developed a condition of gastric dilatation which caused its death in spite of operative attempts to save it.

7. Complete recovery from spastic paralysis was brought about in the remaining male of the litter by similar treatment using a filtrate of the B_1 concentrate. After recovery this rat was again deprived of the B_1 factor for 3 days. On the fourth day immoderate gastric dilatation occurred. Intragastric injection of the antineuritic filtrate caused complete alleviation of this condition within four hours. This animal recovered entirely when continued on B_1 and B_2 in adequate amounts.

8. One female of the litter, although slightly paralyzed, recovered spontaneously and was weaned.

9. No female of the litter showed gastric dilatation.

10. Gastric dilatation was found in 3 of the 4 males.

It would appear that we were dealing with a condition of pylorospasm, probably brought about by a lack of vitamin B_1 , as a complete cure was occasioned by treatment with our B_1 preparation.

We present this study in the hope of stimulating the interest of other workers in this line of research.

BIBLIOGRAPHY

- (1) MOORE, BRODIE AND HOPE. *This Journal*, 1927, lxxxii, 350.
- (2) OSBORNE AND MENDEL. *Journ. Biol. Chem.*, 1922, liv, 739.
- (3) ANDEREGG, L. T. *Journ. Biol. Chem.*, 1924, lix, 587.
- EVANS, H. M. AND H. S. BURR. *Journ. Biol. Chem.*, 1928, lxxvi, 263.
- GRANT, A. H. *Univ. Cincinnati Med. Bull.*, 1924, iii, 17.
- HARTWELL, G. A. *Biochem. Journ.*, 1921, xv, 140; 1925, xix, 1075.
- HELLER, V. G. *Journ. Biol. Chem.*, 1923, lv, 385.
- HOGAN, G. A. AND H. M. HARSHAW. *Journ. Met. Res.*, 1924, v, 3.
- MANVILLE, I. A. *Science*, 1926, lxiv, 256.
- MATTILL, H. A. AND N. C. STONE. *Journ. Biol. Chem.*, 1923, lv, 443.

- (3) NELSON, P. M. *Journ. Home Econ.*, 1926, xviii, 7.
NELSON, V. E., JONES, HELLER, PARKS AND FULMER. *This Journal*, 1926, lxxvi, 325.
SURE, B. *Journ. Amer. Med. Assoc.*, 1927, lxxxix, 675; *Journ. Biol. Chem.*, 1927, lxxiv, 55; *Proc. Amer. Soc. Biol. Chem.*, *Idem.*, 1927, lxxiv, 681.
SURE, B. AND S. J. SCHILLING. *Amer. Journ. Dis. Child.*, 1928, xxxv, 811.
- (4) BRODIE, J. L. *This Journal*, 1929, lxxx, 340.
- (5) MOORE, BRODIE, DENNIS AND HOPE. *Arch. Ped.*, 1929, xli, 416.
- (6) KOPLIK, H. *Amer. Journ. Med. Sci.*, 1908, cxxxvi, 1.
PEHU, M. ET X. PINEL. *Le Nourrison*, 1921, lx, 337.
- (7) MOORE, PLYMATE, ANDREW AND WHITE. *This Journal*, 1932, cii, 573.
- (8) MOORE, PLYMATE AND ANDREW. *This Journal*, 1932, cii, 581.
- (9) HAUGE AND CARRICK. *Journ. Biol. Chem.*, 1926, xix, 463.

THE EFFECT OF COELIAC GANGLIONECTOMY ON THE SUGAR TOLERANCE OF DOGS¹

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The nervous regulation of carbohydrate metabolism has been reviewed by Pollack (1923) and Macleod (1931). More recent observations following stimulation and section of the vagus and section of the splanchnic nerves have been summarized by us in another article (in press). These contributions indicate that 1, certain types of vagal stimulation produce hypoglycemia, presumably through increased secretion of insulin, and 2, that section of the splanchnic nerves increases the susceptibility of the animal to insulin. In the light of these data it seemed important to examine the effect of coeliac ganglionectomy on sugar tolerance, especially so since previous data were not available in the literature.

METHODS OF EXPERIMENTATION. In a previous article (1930) we have described various trials with oral, intraperitoneal and intravenous administration of dextrose for purposes of determining the sugar tolerance of dogs. By far the most constant data were obtained by the method of Woodyatt, Sansum and Wilder (1915). We have insisted on a possibly complete relaxation of the animal, which was trained to lie quietly in a basal state, during an hour of injection. Excitable dogs, or dogs that were difficult to train to lie quietly for an hour were rejected. "Pfanstiehl" dextrose was used in 5 per cent solution, autoclaved and driven through glass coils immersed in a water bath, so that the solution was introduced at approximately body temperature. The dextrose solution was injected with the help of the Woodyatt pump at timed rates for the period of an hour. A twenty-four hour sample of urine was collected under thymol and the sugar, if present, was determined by Benedict's method. From two to three pre-operative tolerances were determined which checked within a 0.1 gram of dextrose per kilogram body weight. The tolerance was expressed as the maximal amount of dextrose that could be injected during the period of an hour without producing a glycosuria. Post-operative determinations were made at the end of two weeks and subsequently once a

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month. The dogs were observed for at least six months, but some have been followed for over two years.

In some of the earlier experiments blood sugar determinations were also made before, at the end and for several hours after the intravenous injection in order to study the rate of disappearance of the excess of dextrose from the blood stream.

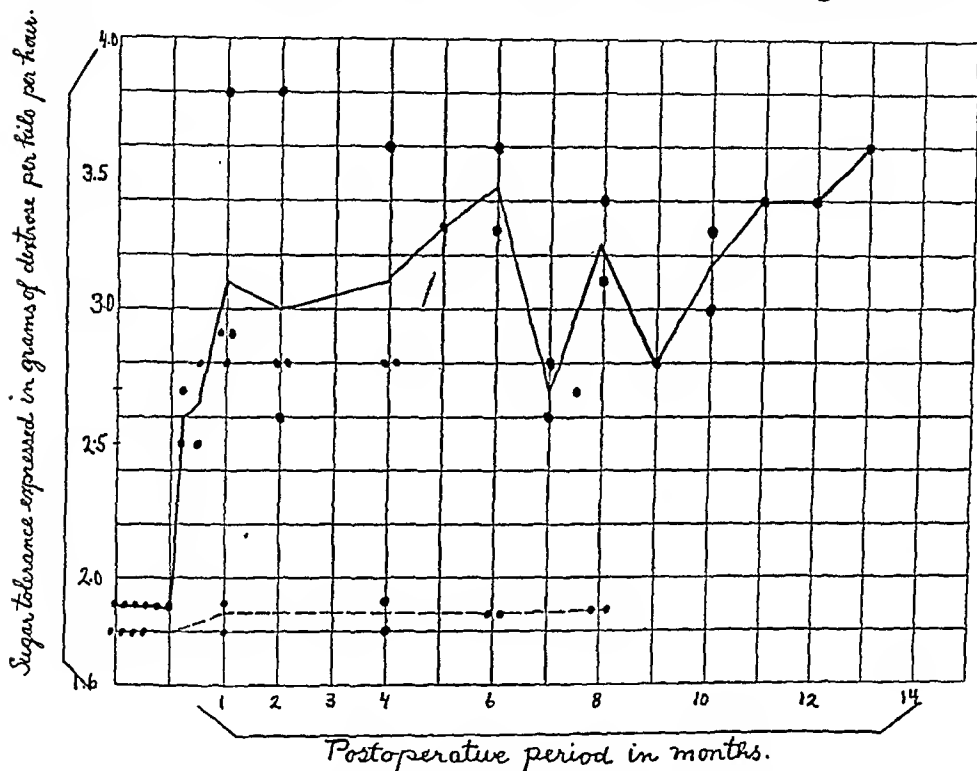
In addition the response of these dogs to insulin was studied before and after operation. Insulin was given in doses of $\frac{1}{10}$ of a unit per kilogram dissolved in 1 cc. of normal salt solution. Blood sugars were determined before, and 10, 20, 30 minutes, 1 and 2 hours after the injection.

The technique of operation has undergone several modifications since the experiments were started. The semilunar ganglia can be reached through an incision which runs parallel to the right or left intercostal arch. When the operation is started from the left side, the suprarenal plexus is picked up just mesial to the left adrenal gland and followed toward the midline, until around the coeliac axis, the left coeliac ganglion and on the other side of the artery, the right coeliac ganglion is reached. Or one can start from the right side, expose and retract the vena cava to the right and remove the coeliac ganglia from the right side. The ganglia can be very definitely recognized by their somewhat different color, which distinguishes them from the subordinate plexuses. Their shape and size, as in man, are variable. The ganglia have to be dissected off bluntly from the coeliac axis, which is comparatively long in the dog. It has been our impression that the most complete excision of these variable structures can be accomplished by exposing them, through one incision, from both sides, thoroughly dividing all connections between the ganglia and the adrenals.

RESULTS. A total of ten dogs was studied before and after coeliac ganglionectomy. The sugar tolerance of normal dogs, determined by the method described above, varied only very slightly between 1.8 and 1.9 gram of dextrose per kilogram per hour. Following the operation, a rise in tolerance occurred in every instance varying, however, between the minimum of 2.8 grams to the maximum of 3.8 grams of dextrose per kilogram body weight. While a rise was present at the first post-operative determination, which occurred at the end of two weeks, there was a tendency to a further rise in tolerance, which seemed to reach its peak at the sixth month. From then on it would fluctuate slightly, influenced by respiratory or skin infections, but generally remained high as long as determinations were made. While the surgical mortality was small, we lost several dogs months later by distemper, so that their data could only be partially utilized. In graph 1, all pre- and post-operative tolerance determinations (40 in number) have been charted. An average curve shows the decided and persistent rise in tolerance following coeliac ganglionectomy. There are considerable variations in the responses of the individual dog.

Two dogs, whose liver had been denervated, do not show a rise in tolerance at any time, in spite of the fact that six post-operative determinations have been made on each animal. These were used as controls.

A study of the blood sugar levels at the end and for four hours after the infusion of 2 grams of dextrose per kilo, shows that in the normal animal, the average blood sugar rose to slightly over 300 mgm. per 100 cc. at the end of the dextrose infusion. An hour later, the blood sugar was normal. Following coeliac ganglionectomy, the peak of the blood sugar level was



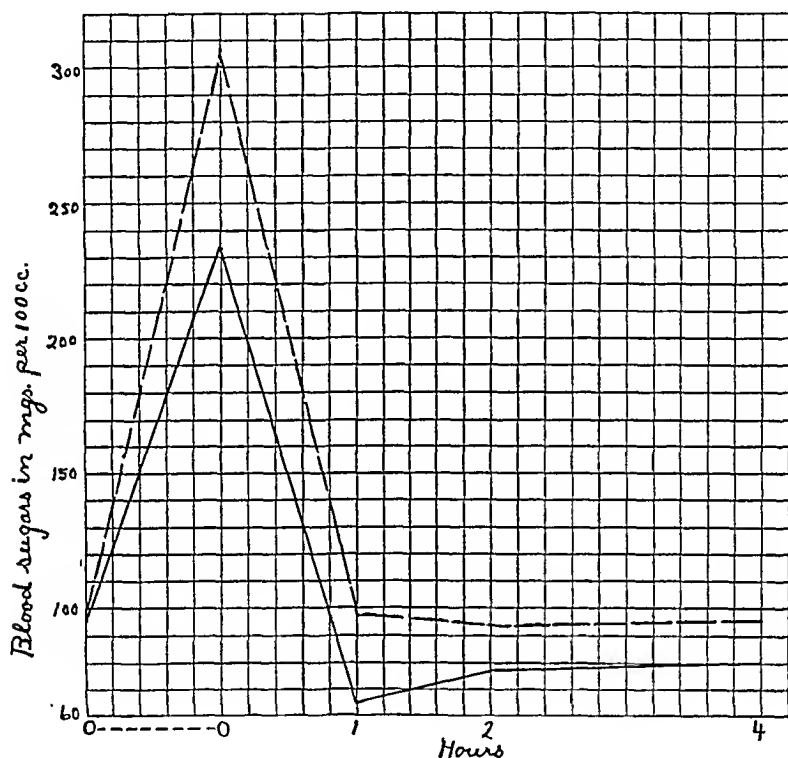
Graph 1. Average curve of the rise in sugar tolerance following coeliac ganglionectomy. An immediate rise takes place following the operation which persists with some fluctuations at the end of a year. The sugar tolerance is expressed in grams of dextrose per kilo per hour. The control curve shows the effect of liver denervation on sugar tolerance.

70 mgm. lower, and a glycosuria did not develop. The blood sugar dropped to a hypoglycemic level at the end of one hour, and returned to normal at the second hour. The rate of disappearance of the injected dextrose from the blood stream is obviously increased after the operation (graph 2).

The response of the operated animals to insulin is definitely increased. The dose of 0.1 unit per kilogram body weight was so selected that it would produce a small dip in the blood sugar level of the normal animal.

Following coeliac ganglionectomy, this dose would produce not only a marked hypoglycemia but quite a delay in the return to the normal level. (table 1).

COMMENT. The method of determining sugar tolerance by an intravenous injection of dextrose at timed rates has given consistent results in our hands. In over fifty determinations on normal dogs, which were made in this series and in other experiments, the rate of utilization proved to be between 1.8 and 1.9 gram of dextrose per kilogram per hour. These figures are much higher than those determined by the originators of the



Graph 2. The effect of coeliac ganglionectomy on the rate of disappearance of injected dextrose from the blood stream.

0-----0: 2 grams of dextrose per kilo were given intravenously for one hour.

Interrupted line: before coeliac ganglionectomy, average of 8 determinations.

Straight line: after coeliac ganglionectomy, average of 5 determinations.

method (Woodyatt, Sansum and Wilder, 1915). We have trained the dogs to permit us a determination in a basal, relaxed state of the animal and we have used "Pfanstiehl" dextrose in a 5 per cent, and in some of the experiments in a 10 per cent solution. All these factors influence the rate of utilization.

Control tolerance determinations, repeated from two weeks to a month, did not raise the tolerance of the dog at any time above 2 grams of dextrose

per kilo per hour. However, following coeliac ganglionectomy, the tolerance rises as early as the first week after the operation, when the dog has not even completely recovered. At the end of a month, the rate of utilization rises to about 3 grams of dextrose per kilo per hour and from then on shows a slight tendency to rise. It has never been observed to drop back

TABLE 1

Showing the increase in sensitivity to insulin which occurs in dogs after removal of the coeliac ganglion

The dogs were given 0.1 unit of insulin per kilogram body weight, dissolved in 1 cc. of normal salt solution, intravenously.

DOG NUMBER	POST-OPERATIVE PERIOD	FASTING BLOOD SUGAR	10 MINUTES	20 MINUTES	30 MINUTES	1 HOUR	2 HOURS
Insulin response following coeliac ganglionectomy							
62	5 days	89	64	46	41	60	91
87	2 weeks	80	71	58	48	64	80
85	2 weeks	70	51	46	46	54	70
86	2 weeks	68	64	46	40	45	68
68	5 weeks	67	68	67	53	46	67
73	2 months	87	83	54	44	62	87
75	2 months	75	74	53	47	41	84
77	2 months	70	58	49	46	44	69
78	2 months	75	66	52	40	38	60
56	4½ months	87	48	55	64	89	113
Average values.....		76.8	64.7	52.6	46.9	54.3	78.9
Insulin response of normal dogs							
66		101	90	85	79	87	97
65		98	92	74	68	78	90
64		101	98	72	76	102	95
48		83			76	70	76
50		90			83	75	80
51		87			85	70	73
101		82	86	61	63	72	81
102		71	68	57	57	64	71
103		70	61	53	57	51	71
104		82	65	50	52	68	82
Average.....		93.5	80	64.5	69.6	73.5	81.6

to the pre-operative level, although we have studied one dog for sixteen months.

When the dextrose is injected into the blood stream, the hyperglycemia reaches a lower level after the operation and reacts with a definite hypoglycemia. Whether this means that more insulin is available or that the animal is more sensitive to the same amount of insulin cannot be stated

on the basis of the present data. It appears that the action of extraneous insulin is exaggerated in the ganglionectomized dog. In fact, minute doses, such as 0.1 unit of insulin per kilogram body weight given intravenously, are capable of producing a hypoglycemia of 40 milligrams and less with a visible reaction, following coeliac ganglionectomy. We believe, however, that further experimentation is necessary to establish the value of this method for determining insulin susceptibility.

The mechanism of the action of coeliac ganglionectomy in augmenting sugar "tolerance" needs further analysis. A denervation of the liver, as shown by Donald (1931) and our few observations, does not produce a rise in tolerance. There remains then the action on the pancreas and adrenals. It is more than likely that coeliac ganglionectomy will increase the blood flow to the pancreas, although this problem needs further investigation (1931). It may interrupt vasoconstrictor impulses to the vascular network of the islets. In preliminary tests, adrenal denervation alone has also given a marked rise in tolerance, so that coeliac ganglionectomy may operate in that manner. Even though it may not be clear at the present time how removal of the coeliac ganglion leads to an increase in sugar "tolerance" our data yield additional evidence to that already recorded in the literature within recent years, showing that nervous factors (glyco-regulatory impulses) influence insulin production or requirement.

SUMMARY

The effect of coeliac ganglionectomy on the sugar tolerance of normal dogs was studied. The intravenous sugar tolerance of normal dogs does not vary spontaneously under basal conditions. The removal of the coeliac ganglion results in a decided, persistent rise in tolerance in every instance. Intravenously administered dextrose disappears more rapidly from the blood stream than before the operation. The dogs become more susceptible to insulin. Denervation of the liver does not have a similar action. These data show that the coeliac ganglion mediates nerve impulses, the exclusion of which brings about either an increased insulin production or a reduction in the insulin requirement.

BIBLIOGRAPHY

- POLLACK, L. 1923. *Ergebn. d. inn. Med. und. Kinderh.*, xxiii, 337.
 MACLEOD, J. J. R. 1931. *Lancet*, cccix, 512.
 DE TAKATS, G. AND F. P. CUTHBERT. 1932. *Arch. Surg.* (in press).
 DE TAKATS, G., F. HANNETT, D. HENDERSON, AND I. J. SEITZ. 1930. *Arch. Surg.*, xx, 866.
 WOODYATT, R. T., W. D. SANBURN, AND R. M. WILDER. 1915. *Journ. Amer. Med. Assoc.*, xlv, 2067.
 DONALD, J. M. 1931. *This Journal*, xcvi, 605.
 CAPORALE, L. AND DE FERMO. 1931. *Arch. ital. di chir.*, xxx, 422.

BLOOD URIC ACID FOLLOWING THE INTRAVENOUS ADMINISTRATION OF URIC ACID IN NORMAL DOGS

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The blood and urine of normal dogs contain only minute amounts of uric acid. Following intravenous administration, uric acid disappears from the blood with great rapidity but only a small fraction of the amount given is excreted in the urine. This is apparently true of all breeds of dogs except the Dalmatian. The studies of Mann and Magath (1) and of Bollman, Mann and Magath (2) have shown very conclusively that the liver is the organ primarily responsible for the destruction of uric acid in dogs. Following removal or severe damage of the liver, in dogs, uric acid appears spontaneously in the blood and urine, and parenterally administered uric acid can be quantitatively accounted for in the blood and urine. The exhaustive studies of Folin, Berglund and Derick (3) show that uric acid does not diffuse readily into the general body tissues. Hunter and Givens (4) clearly demonstrated the relationship between the excretion of uric acid and of allantoin. It is now generally believed that in dogs most of the uric acid, of either endogenous or exogenous origin, is oxidized to allantoin and excreted as such.

It follows from the above facts that in dogs the disappearance of intravenously administered uric acid from the blood should be a rather accurate index of the rate at which uric acid is destroyed or oxidized to allantoin.

The results reported in this paper are the first of a series of experiments planned to study uric acid metabolism in dogs and human subjects by means of the blood uric acid tolerance curves following intravenous administration of uric acid.

METHODS AND PROCEDURE. All of the experiments reported in this paper were performed upon the same dog—a mongrel male weighing approximately 12.2 kilos. During the period of about eight months occupied by the experiments the animal was maintained upon a low purine diet consisting of fresh whole milk and karo syrup in quantities sufficient to cover the energy requirements. The animal was routinely fed at 4:30 p.m. each day. On days preceding experiments no food was given but water was allowed ad libidum. During the entire experimental period the

animal was kept in a small individual metabolism cage in warm animal quarters. During the early part of the work the animal developed distemper but after a period of approximately two weeks made an uneventful recovery. With this exception the animal remained in good health and showed a definite gain in weight during the experimental period.

The urine collections were carried out as follows: About 2:00 p.m. on the day preceding the experiments, the bladder was emptied by catheter and the animal placed in a metabolism cage. The following morning, just before the intravenous injection of uric acid, the bladder was emptied by catheter and the urine added to the amount already collected. Following the injection of uric acid, the urine was collected by catheter every two or three hours for the next six or eight hours, after which the animal was placed in a metabolism cage and the urine collected for the remaining part of the twenty-four hour period at the end of which the bladder was again emptied by catheter.

Blood uric acid determinations were made by the new method of Folin (6) using unlaked blood (5). As pointed out by Folin, this method is very accurate and is free from practically all of the errors inherent in the older methods.

The uric acid was administered intravenously in a solution originally recommended by Koehler (7). This solution contains 1.0 gram of uric acid, 0.28 gram of lithium carbonate and 1.35 gram of glucose per 100 cc. By increasing the lithium carbonate in proportion to the uric acid, we were able to obtain a solution containing as high as 1.2 gram of uric acid per 100 cc.

When single intravenous injections of uric acid were given the required amount of the solution was injected into the jugular vein. The time occupied by the injection was usually from one to three minutes. Blood samples were then collected at stated intervals from the opposite jugular vein.

When continuous uric acid injections were employed the solution was injected into the saphenous vein by means of a simple gravity controlled injection apparatus which permitted the flow per minute to be accurately regulated. Blood samples were collected from the jugular vein.

RESULTS. All experiments performed may be divided into three groups as follows:

I. *Single injections.* In these experiments the uric acid was given in a single injection. The total quantities of uric acid injected in the various experiments were 0.640, 0.860, and 1.20 gram. The time required for the injection varied from one to three minutes and following the injection blood samples were drawn as rapidly as possible for varying periods of time.

II. *Continuous injections.* In these experiments the uric acid solution was injected into the saphenous vein as described above. The total

quantities of uric acid injected in the various experiments were 1.20 gram; 1.65 gram and 1.80 gram. The rate of injection was regulated so that the amount of uric acid administered was 10 mgm. per minute. This rate of injection was maintained for periods of two to three hours in the various experiments. Blood samples for analysis were obtained from the jugular vein during the continuous injection.

III. *Combined single and continuous injections.* In these experiments the continuous injection was given into the saphenous vein as described above. The rate of the continuous administration was 10 milligrams of uric acid per minute. The continuous injection was maintained throughout the duration of the experiment. About 45 to 60 minutes after the start of the continuous injection, a single injection was given into the jugular vein in the usual manner. The amount of uric acid given in the single injection was usually 0.8 gram. This amount was injected in from one to two minutes.

Each of the above types of experiments was repeated many times and the outstanding feature of the results was the remarkable agreement between the individual experiments in each type. In reporting the results one curve illustrating each type of experiment will be given and any important variations in other experiments will be mentioned.

(A) *Single injections.* A total of ten experiments was performed in which the uric acid was given in a single injection. Figure I shows the blood uric acid curve following a single injection of 0.640 gram of uric acid. In analyzing this curve it was found that when the logarithm of the milligrams of uric acid per 100 cc. was plotted against the time after injection the result was a straight line (fig. II). The rate of decrease is about 3 per cent of the amount present per minute. Figure III shows the blood uric acid curve following a single injection of 1.20 gram of uric acid. In this experiment the blood uric acid was determined until it reached 0.7 mgm. per 100 cc. In order to determine such small quantities, the usual quantity of the protein-free filtrate was made up to a final volume of 12.5 cc. instead of the usual 25 cc. Figure IV shows the result of replotting this curve with the logarithms of the concentrations of the blood uric acid as the ordinates and the time after injection as abscissae. It is seen that the blood uric acid decreases in a straight line for 57 minutes after the end of the injection, the rate of decrease being 4.5 per cent of the amount present per minute; after 57 minutes, or when the concentration of blood uric acid becomes less than 1.5 mgm. per 100 cc., there is a break in the curve and the rate of disappearance of uric acid from the blood drops to about 1.7 per cent of the amount present per minute. This break in logarithmic curves when the concentration of the reacting substances becomes very low is a common occurrence. We have no experimental proof as to its cause in these experiments but at least three possible ex-

planations may be mentioned: first, it may signify that there are two processes concerned in the disappearance of the uric acid from the blood one of which is much more rapid than the other, in this case, the first part of the curve would represent the sum of both processes with the more rapid process predominating while the second part of the curve would represent the slower process; second, in vitro experiments on enzyme actions usually show a decrease in the rate of transformation when there is an accumulation of the end products of the particular enzyme reaction; while it is not impossible for this to occur in the intact animal, it nevertheless appears quite unlikely; third, with very low concentrations the rate of disappearance may be secondarily governed by the rate of blood flow through the organs concerned with the disappearance of uric acid from the blood, this factor being relatively unimportant when the concentration of uric acid in the blood is high. Our data do not enable us to distinguish between these possibilities.

All of the single injection experiments performed gave results similar to those shown in figures I to IV. Following the injection the blood uric acid decreased logarithmically; when the concentration became as low as 1.5 to 2.0 mgm. per 100 cc. there was a decrease in the rate of disappearance. The rate at which the uric acid disappeared from the blood, before the break in the curves, varied between 6.5 per cent and 3 per cent of the amount present per minute; after the break the rates of disappearance varied between 3.3 and 0.9 per cent per minute. There was relatively little difference in the rates of disappearance from the blood when the quantity of uric acid injected varied from 0.640 to 1.20 gram. In one experiment performed while the animal was acutely ill with distemper in which 0.640 gram was injected, the rate of disappearance from the blood was only 1 per cent of the amount present per minute. This was the slowest rate of decrease in blood uric acid noted in any of the experiments. An experiment performed about one month later, after complete recovery from distemper, and in which the same quantity was injected showed a decrease of 3 per cent per minute which was well within the normal range. The effect of fasting for 5 days and of feeding a meal of bread, karo syrup and milk one-half hour before the experiment were tried but neither of these procedures caused significant alteration in the results.

The per cent of the uric acid which was excreted in the urine in these experiments was very small and varied between a maximum of 5.5 per cent in an experiment in which 0.640 gram was injected, and a minimum of 2.7 per cent with injection of the same quantity. The average for all experiments was 4.1 per cent of the amount injected. The actual quantities excreted varied from 0.023 gram to 0.044 gram when the amount injected was 0.640 and 1.20 gram respectively. From these figures it is evident that the amount excreted in the urine was negligible. From 95

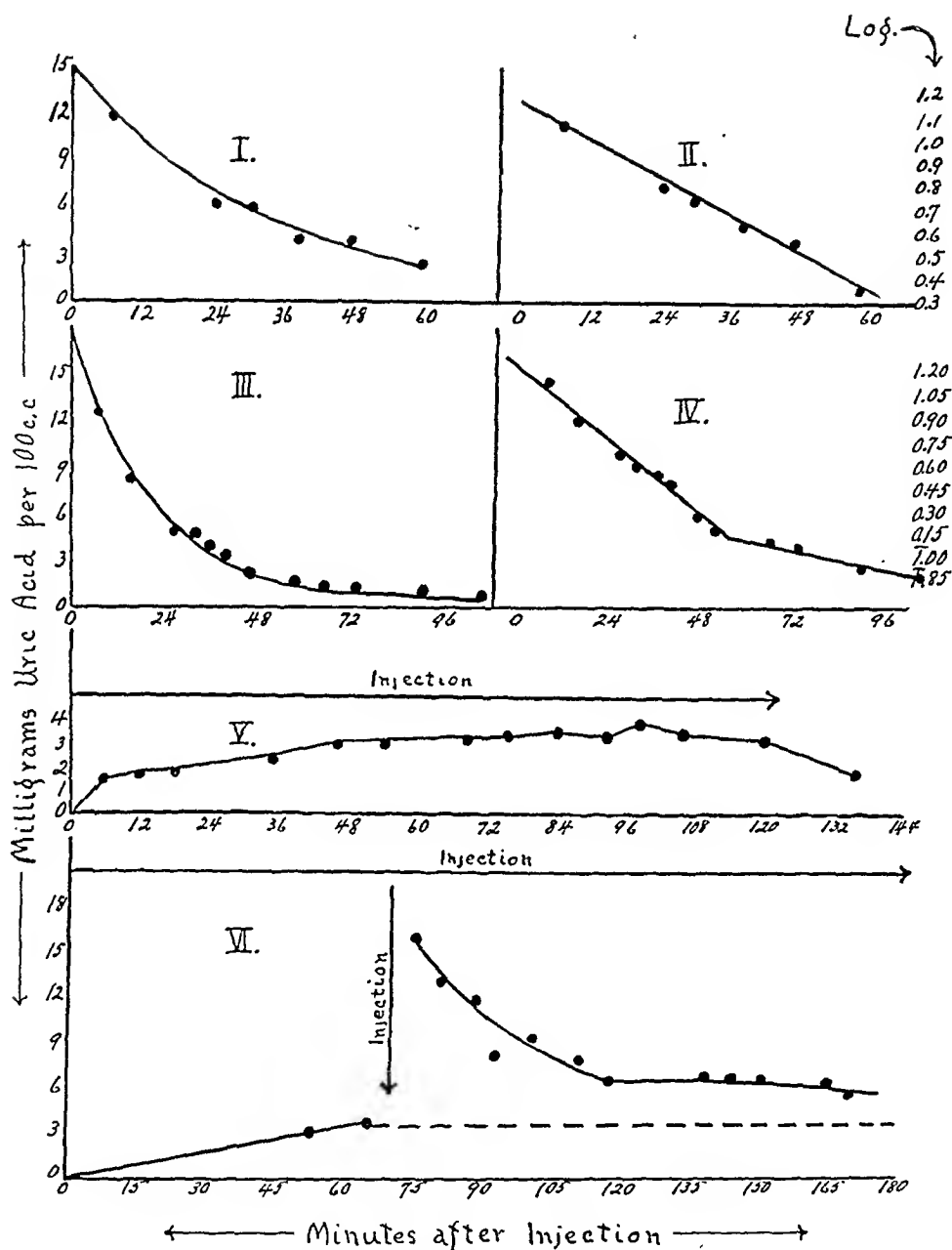


Fig. I. The blood uric acid following a single intravenous injection of 0.640 gram of uric acid. Five and five-tenths per cent of the amount injected was excreted in the urine.

Fig. II. The curve shown in figure I replotted with the logarithm of the blood uric acid concentration against the time after injection. The rate of disappearance is approximately 3 per cent per minute.

Fig. III. The blood uric acid following a single intravenous injection of 1.20 gram of uric acid. Three and seven-tenths per cent of the amount given was excreted in the urine.

to 100 per cent of the total quantity excreted in the urine was secreted during the first three or four hours after injection. In two experiments the urine was collected, by catheter, every two hours for twenty-four hours after the injection in order to be certain that there was not a second period of increased excretion, but it did not occur in either experiment.

The results of these single injection experiments show quite clearly that when quantities of uric acid varying from 0.640 to 1.20 gram were administered to normal dogs, very little was excreted in the urine and that the blood uric acid follows a logarithmic curve, the rate of decrease being approximately 3 per cent of the amount present per minute.

(B) *Continuous injection.* In these experiments the solution injected usually contained 10 mgm. of uric acid per cubic centimeter and was injected into the saphenous vein at a rate of approximately 1 cc. per minute. Blood samples for analysis were withdrawn from the jugular vein. Figure V shows a typical experiment in which the injection was continued for 123 minutes. Other experiments were continued for as long as three hours. The results were strikingly similar in all experiments. The blood uric acid began to rise almost immediately after the start of the injection and usually reached its maximum height in from 45 to 60 minutes after the start of the injection, from this time on to the end of the injection a steady state was maintained in which the blood uric acid was very constant. In five such experiments the value of the blood uric acid during the steady state averaged from 2.8 to 3.3 mgm. per 100 cc. and this level was maintained as long as the injection was continued. As soon as the injection was discontinued the blood uric acid began to fall but the decrease was usually very slow. In one experiment in which the injection was maintained for 183 minutes, the blood uric acid dropped from a steady state of 3.3 mgm. per 100 cc. to 1 mgm. per 100 cc. during a period of 63 minutes after discontinuing the injection.

The quantity of uric acid excreted in the urine during the continuous injection was definitely greater than in the single injection experiments and varied between 8 and 9 per cent of the quantity injected or in actual

Fig. IV. The curve shown in figure III replotted with the logarithm of the blood uric acid concentration against the time after injection. From 0 to 57 minutes rate of disappearance is approximately 4.5 per cent per minute; from 57 to 106 minutes the rate of disappearance is approximately 1.7 per cent per minute.

Fig. V. Continuous injection of uric acid at the rate of 10 mgm. per minute. The steady state reached after 46 minutes averages 3.3 mgm. per 100 cc. A total of 1.20 gram was injected and 8 per cent was excreted in the urine.

Fig. VI. A continuous injection at the rate of 10 mgm. per minute maintained for 183 minutes. A single injection of 0.8 gram given 72 minutes after the start of the continuous injection. Dotted line shows the probable level of the blood uric acid as maintained by the continuous injection. Approximately 36 per cent of the 0.8 gram given in the single injection was excreted in the urine.

amounts from 0.096 to 0.150 gram. The greatest excretion usually occurred during the period of injection and only small quantities were excreted after the injection was discontinued.

The very slow return of the blood uric acid to the zero level following the cessation of the injection and the greater amount excreted in the urine both suggest that the paths of uric acid destruction are possibly clogged by a continuous injection over long periods of time. In some but not all of the continuous injection experiments the animal became nauseated and vomited during the injection. The vomitus was found to contain uric acid.

(C) *Combined single and continuous injections.* Since it was possible to maintain the blood uric acid at a constant level of approximately 3.2 mgm. per 100 cc., by means of a continuous injection, it was decided to study the result of a combined continuous and single injection. An example of this type of experiment is shown in figure VI. The continuous injection was usually maintained for about one hour before the single injection was given, at which time the blood uric acid had usually reached the steady state at a level varying between 3 and 4 mgm. per 100 cc. At this time the single injection of 0.8 gram of uric acid was given into the jugular vein. The continuous injection was maintained during the entire experimental period. In figure VI the probable level of the steady state has been represented by the dotted line at 3.3 mgm. of uric acid per 100 cc. Following the single injection of 0.8 gram of uric acid the blood uric acid rose to 15.7 mgm. per 100 cc. and then began to fall rapidly, the general exponential nature of the curve being similar to the curves shown in figures I and III. The rate of the decline for 45 minutes after the single injection was approximately 2 per cent of the amount present per minute, which is only slightly slower than the rate in the ordinary single injection experiments. It is important to note, however, that following the single injection the blood uric acid did not return to the predicted basal level, since at 98 minutes after the single injection the blood uric acid was still 2.5 mgm. above the theoretical basal level of 3.3 mgm. per 100 cc. maintained by the continuous injection. This delayed return is emphasized when this experiment is compared with the experiment shown in figure III in which the blood uric acid had decreased to less than 1 mgm. 98 minutes after a single injection of 1.20 gram of uric acid.

It was rather difficult to calculate exactly how much of the 0.8 gram given in the single injection was excreted in the urine. However, by averaging the per cent excreted in several continuous injection experiments and subtracting this amount from the total excreted in the combined continuous and single injection experiments we were able to calculate that from 20 to 36 per cent of the 0.8 gram given in the single injection was excreted in the urine. This is very greatly in excess of the amount excreted in the ordinary single injection experiments.

In these experiments the dog usually became nauseated and vomited following the single injection. The vomitus was a mucoid bile stained fluid and was found to contain uric acid.

DISCUSSION. It is not surprising to find that the decrease in blood uric acid following a single intravenous injection is a logarithmic curve corresponding to a monomolecular reaction. As was mentioned in the introduction, it is now generally believed that in the dog most of the uric acid is oxidized to allantoin. Folin and Berglund have shown that uric acid does not readily diffuse into the general body tissues and Mann and his co-workers have definitely shown that the liver is the sole organ concerned in the oxidation or destruction of uric acid. Since only very small quantities of the uric acid were excreted in the urine, it is very reasonable to suppose that the nature of the blood uric acid curves is indicative of a monomolecular reaction in which one molecule of uric acid is oxidized to one molecule of allantoin.

The results obtained with continuous injections of uric acid are very interesting and show that the ability of the dog to destroy uric acid is sharply limited. When the rate of inflow of uric acid exceeds the maximal rate at which uric acid can be oxidized it accumulates in the blood. With the dog used in these experiments the injection of 10 mgm. of uric acid per minute resulted in a steady state of the blood uric acid level which could be maintained at about 3.3 mgm. per 100 cc. for as long as three hours, thus indicating that the rate of inflow was exceeding the rate of destruction but that both were constant. It is quite likely that different dogs would show differences in the rate of injection necessary to maintain the steady state.

When a single injection was combined with a continuous injection, the decrease of the blood uric acid following the single injection was a logarithmic curve but it failed to return to the predicted basal level as was the case in simple single injection experiments; in most of the experiments it was from 2 to 3 mgm. above the predicted basal level at the end of one and one-half to two hours. This behavior of the blood uric acid is very similar to that observed in the human subject following a single intravenous injection of uric acid in which the blood uric acid often decreases in a logarithmic curve for a short time, but the decrease soon stops and the blood uric acid may then remain above the normal pre-injection level for a considerable period of time. This finding suggests that the difference between the dog and the human may be quantitative rather than qualitative. This problem is being investigated in greater detail at the present time.

SUMMARY

1. Following a single intravenous injection of from 0.640 to 1.20 gram of uric acid, the blood uric acid rose to a high level. The disappearance of the uric acid from the blood followed a logarithmic curve suggesting a monomolecular reaction. The uric acid excreted in the urine was small in amount and varied between 2.7 and 5.5 per cent of the amount injected.

2. When uric acid was injected continuously at a rate of approximately 10 mgm. per minute, the blood uric acid rose for about 45 or 60 minutes and then reached a steady state of approximately 3.3 mgm. per 100 cc. This steady state could be maintained, by continued injection, for as long as three hours. In these experiments the amount of uric acid excreted in the urine was about 8 or 9 per cent of the quantity injected.

3. When a single injection of 0.8 gram was given during the course of a continuous injection, the decrease in the blood uric acid following the single injection was a logarithmic curve but there was a delay in the return to the level maintained by the continuous injection. It was calculated that from 20 to 36 per cent of the amount given in the single injection was excreted in the urine.

BIBLIOGRAPHY

- (1) MANN, F. C. AND T. B. MAGATH. *This Journal*, 1921, iv, 286.
- (2) BOLLMAN, J. L., F. C. MANN AND T. B. MAGATH. *This Journal*, 1925, lxxii, 629.
- (3) FOLIN, O., H. BERGLUND AND C. DERICK. *Journ. Biol. Chem.*, 1924, lx, 361.
- (4) HUNTER, A. AND M. H. GIVENS. *Journ. Biol. Chem.*, 1912, xiii, 371; 1914, xviii, 37, 107, 387, 403.
- (5) FOLIN, O. AND A. SVEDEBERG. *Journ. Biol. Chem.*, 1930, lxxxviii, 85.
- (6) FOLIN, O. *Journ. Biol. Chem.*, 1930, lxxxvi, 179.
- (7) KOEHLER, A. E. *Journ. Biol. Chem.*, 1924, lx, 121.

THE EFFECT OF ANOXEMIA ON THE DIGESTIVE MOVEMENTS OF THE STOMACH

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In the course of experiments on the effect of low oxygen tensions on gastro-intestinal motility, we have already reported the effect of anoxemia on hunger contractions (Van Liere and Crisler, 1930). The effects upon the digestive movements of the stomach are given in the present paper. Any relationship between anoxemia and digestive movements will be particularly important in its application to disease associated with low blood-oxygen tensions such as the gastro-intestinal upsets in cardiac disease. But the rising popularity of living at high altitudes as occasioned by aviation has extended the importance of studying anoxemia beyond the scope of disease.

METHODS. Because of the difficulty of recording movements of the well filled stomach by the balloon method we have used the gastrograph. In no animal did we fail to get records by this method but in several cases no record could be obtained with the balloon, presumably because of defects in the transmitting system caused by kinks, etc. We therefore ran just enough by the balloon method to convince us that the two methods run parallel (see fig. 1). The gastrograph method involves opening the abdomen of the animal so that the present experiments, unlike the ones on hunger contractions, were done under light anesthesia. The data were obtained from fourteen dogs fed from fifteen minutes to one hour before anesthetizing. They were quickly etherized and then injected intravenously with 250 mgm. of barbital-Na per kilo. The animals varied in size from 1.3 to 8.1 kilos and were of all ages. The fixed point of the gastrograph was secured to the transverse band on the lesser curvature of the stomach and the movable point to the corresponding region on the greater curvature. The desired oxygen tensions, adjusted by mixing appropriate concentrations of oxygen and nitrogen in an ethylene-oxygen anesthesia machine, were administered to the animal through a modified Kunde muzzle (Kunde, 1923, and Van Liere and Crisler, 1930). Tracings obtained from animals showing skeletal movements were discarded.

RESULTS. Digestive movements were apparent almost always as quickly as the recording apparatus could be adjusted. Whenever the

oxygen tension became lower than 10 per cent there were distinct signs of inhibition in all animals. Some responded to concentrations as high as 12 per cent. The inhibition consisted of a constant decrease in amplitude of contraction with frequently a fall in tone. The changes in tone were

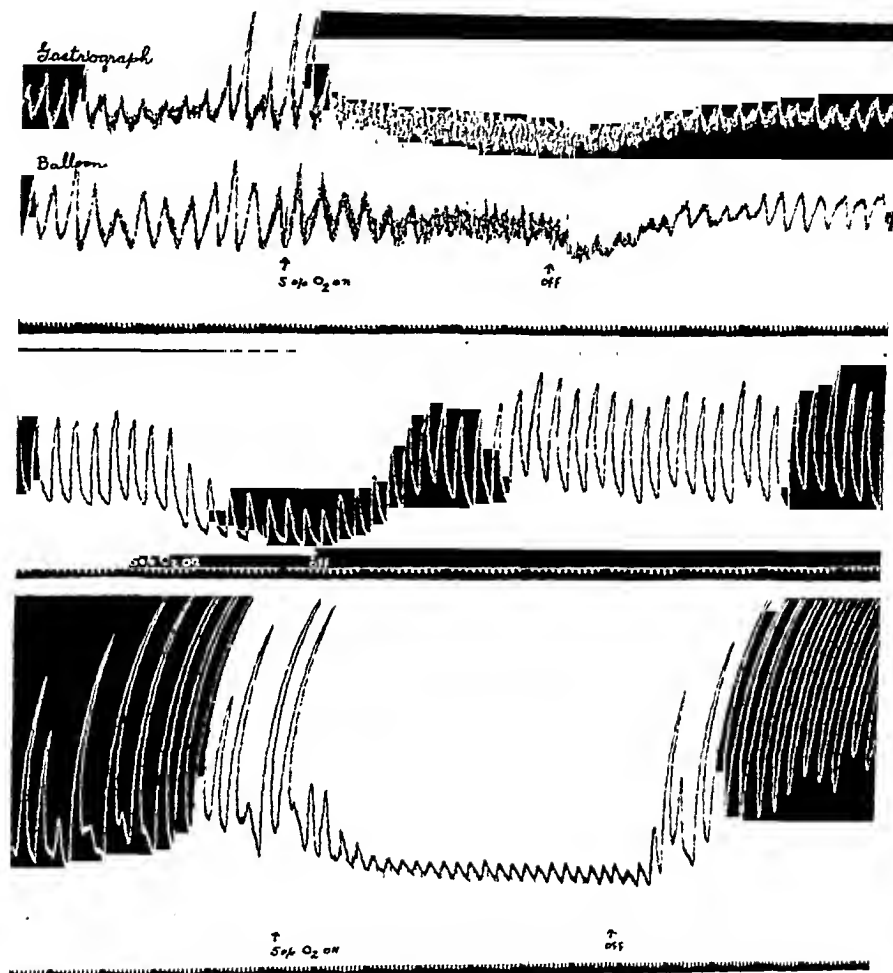


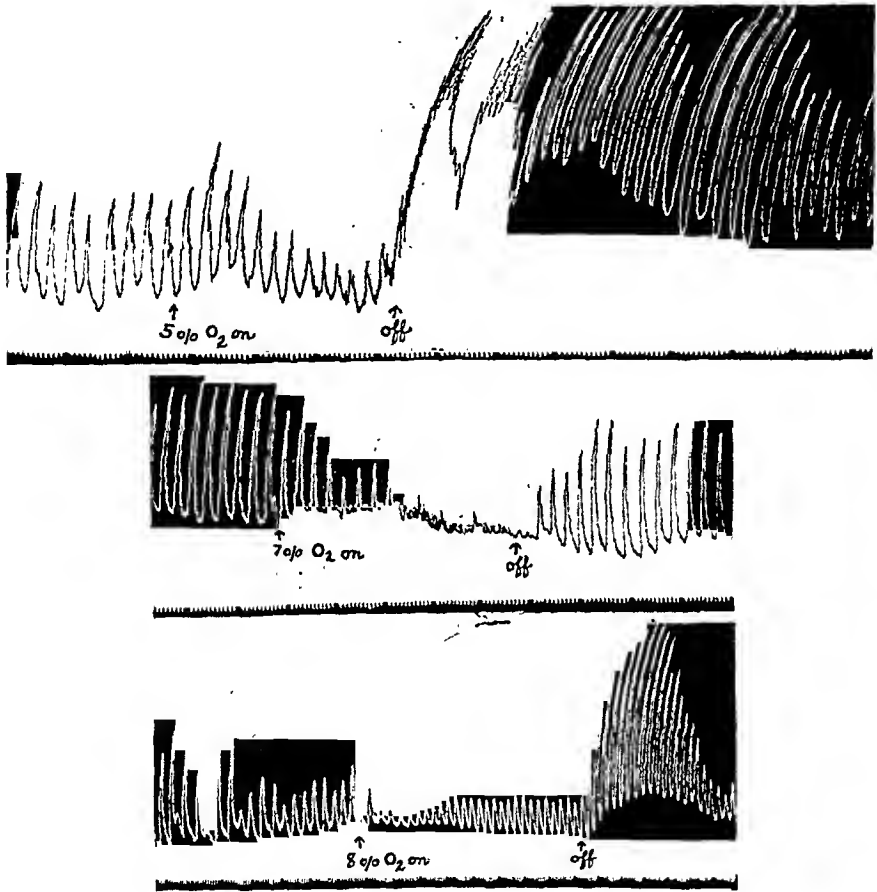
Fig. 1. Simultaneous tracings by gastrograph and balloon to show parallel response by both methods.

Figs. 2 and 3. Response to 5 per cent oxygen in two animals with different amplitudes of contraction and different degrees of tone. Note decrease in amplitude and fall in tone during anoxemia and increase in both to higher than normal levels with stair step effect during recovery.

present when the preceding tone was high, but were absent if the initial tone was sufficiently low (figs. 2 and 6). As the oxygen tension was decreased the inhibition became more nearly complete. In most cases a complete inhibition could be obtained after varying periods with oxygen

tensions below 7 per cent. Occasionally some sort of an "acute tolerance" seemed to develop with a tendency for the contractions to "break through." This was especially noticeable in one animal (fig. 6).

Upon re-oxygenation (allowing the animal to breathe air) there was no post-anoxic depression as in the case of hunger contractions. The



The similar effect of varying percentages of oxygen below 10 per cent.

Fig. 4. Typical response except for some distortion early in the recovery period caused by forced respiration.

Fig. 5. Typical response except that the original tone is so high that the recovery tone does not surpass it.

Fig. 6. Typical response showing a tendency for the contractions to break through while the tone continues to fall during anoxemia.

recovery usually started immediately. If there was a latent period the contractions were of the same nature as those during anoxemia. The recoveries consisted of prompt contractions of successively greater magnitude giving much the same "stair step" effect as seen after the post-anoxic depression in hunger contraction experiments. The amplitude almost in-

variably became greater than normal and there was usually a rise in tone to a level higher than normal. In no case were signs of retching or vomiting seen.

DISCUSSION. The liberation of "sympathin" by anoxemia has been suggested as a possible mechanism in these phenomena. In early experiments on sympathin Cannon and Bacq (1931) were unable to show any effect of sympathin on the denervated intestine, and our experiments yield nothing to substantiate such a mechanism.

A far more plausible explanation involving the sympathetics is that in the early period of anoxemia, with the rise in blood pH from hyper-ventilation, the sympathetics are sensitized so that impulses having no inhibitory effect on normal irritability now become effective. Such an increase in the pH of the blood concomitant with the hyperventilation of the early stages of anoxemia has been demonstrated by Koehler, Behneman, Benell and Loevenhart (1925). Burget and Crisler (1927) have shown that such a sensitization of the sympathetics by a rise in blood pH does exist. Such an explanation may well account for the "breaking through" phenomenon also. This sometimes occurs after two or three minutes and agrees well with the duration of the high pH. After this time the pH begins to fall with a release of the sympathetic sensitization. The breaking through may then be simply a partial escape of the intestines from the sympathetic inhibitory effect. If the anoxemia is prolonged an acidosis will eventually occur from the accumulation of fixed acids. The continued inhibition under these conditions of course points to other causative factors as well as alkalosis.

Another possibility is suggested by the work of McSwiney and Newton (1927b). They demonstrated that alkalosis caused a lowered amplitude of contraction in smooth muscle strips. But in previous experiments (1927a) they found a rise in tone under the same conditions. This does not fit with our results where we got decreased amplitude but a fall in tone. We are inclined to think that our results are not based upon the effect of a higher pH directly on the gut musculature.

Gastro-intestinal upsets in certain cardiac diseases especially have been ascribed to the anoxemia accompanying venous stasis. The decreased motility evident in our graphs may help to explain the constipation in such cases but we were unable to reproduce the retching and vomiting. The anesthesia may have prevented their appearance, or the inconsistencies in the two conditions may be accounted for by the fact that in cardiac disease there is relatively rarely a high pH from hyperventilation.

A paradox would appear to exist between the effect of anoxemia on the tone in hunger contractions and in the digestive movements of the stomach. In hunger contractions the tone seemed to rise while in the digestive movements it fell. The difference in the experimental method was only

in the absence of anesthesia in the former case and its presence in the latter. To test the possible effect of anesthesia we have run control hunger contractions (balloon method) on barbitalized dogs. In these cases a fall in tone occurred as in the case of digestive peristalsis. There is a possibility that the increase in gastric tone reported in our hunger contractions was an artifact from less complete relaxation of the diaphragm during hyperpnea caused by anoxemia. The apparent rise in tone might come from a transmission of the raised intra-abdominal pressure. A greater hyperpnea with less relaxation of the diaphragm and a higher intra-abdominal pressure might easily occur in the unanesthetized dog but disappear in the anesthetized one. It should be reemphasized that all records in which skeletal movements were detected were discarded. The amplitude of contraction, however, was decreased during anoxemia in both cases. There is a lack of parallelism in hunger contractions between the experimental results and the clinical picture because there is not a corresponding decrease in the sensation of hunger as might be expected from the decreased amplitude (Carlson, personal communication). On the other hand, there is an apparent parallelism between gastro-intestinal disturbances in the anoxemia of cardiac disease and the effect of anoxemia on gastric digestive motility.

There is no conflict between our results and the appearance of exaggerated peristalsis, "asphyxial peristalsis," as seen in guinea pigs after a fatal blow on the head. Presumably in the guinea pig so treated the pH of the blood falls from the beginning without the preliminary rise, for the animal stops breathing immediately and the factor of initial hyperventilation is absent, hence, assuming the pH as important in the mechanism, the inhibition also would be absent.

A correlation of these inhibitory effects on gastric motility and the emptying time of the stomach under anoxemic conditions will be treated in another paper.

SUMMARY

Anoxemia of grades of 10 per cent oxygen or less, in barbitalized dogs, causes inhibition of gastric digestive motility as indicated constantly by a decreased amplitude of contraction and frequently by a fall in tone.

The most plausible mechanism for the early inhibition seems to be a sensitization of the sympathetics by the rise in blood pH accompanying the initial hyperpnea and hyperventilation in anoxemia.

These results are a partial reproduction of the clinical picture of gastro-intestinal upsets during venous stasis as seen in cardiac disease. A possible explanation of the discrepancies in the two conditions is mentioned.

BIBLIOGRAPHY

- BURGET, G. E. AND G. CRISLER. 1927. *This Journal*, lxxxiii, 373.
CANNON, W. B. AND Z. M. BACQ. 1931. *Ibid.*, xcvi, 392.
KOEHLER, A. E., H. M. F. BEHNEMAN, O. E. BENELL AND A. S. LOEVENHART. 1925.
Ibid., lxxiv, 590.
KUNDE, M. M. 1923. *Journ. Met. Res.*, iii, 399.
McSWINEY, B. A. AND W. H. NEWTON. 1927a. *Journ. Physiol.*, lxiii, 51.
1927b. *Ibid.*, lxiv, 144.
VAN LIERE, E. J. AND G. CRISLER. 1930. *This Journal*, xciii, 267.

THE EFFECT OF SMALL QUANTITIES OF GALACTOSE ON THE HUMAN RESPIRATORY EXCHANGE

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The effect of the ingestion of galactose on the respiratory quotient and the total metabolism has been determined by Lusk (1915) and Wierzechowski (1931) on dogs and by Deuel (1927) and Cathcart and Markowitz (1927) on men. The amounts of sugar used were 50 and 75 grams with men, and 50 grams and 2 grams per hour per kilo for 3 hours with dogs. As galactose produces a marked change in the respiratory quotient in men with the above quantities, it would seem as though it would be a suitable substance with which to study the effect of varying amounts of sugar on the respiratory quotient. In addition the interest in the metabolism of galactose is increasing, as shown by the number of papers on the effects of ingestion of this sugar. It is well known that there is a lower tolerance for galactose than for either glucose or fructose, and partly for this reason smaller quantities are used than is customary with the other hexoses. Carpenter and Fox (1930a, b) found that there were significant changes in the respiratory quotient and total metabolism after the ingestion of 10 grams or more of either fructose or glucose. We have conducted similar experiments with galactose for the purposes of determining whether the effects upon the respiratory quotient and the total heat production were proportional to the amounts ingested, and of determining how these results compared with those obtained on the influence of the ingestion of glucose and fructose.

METHOD OF DETERMINING THE RESPIRATORY EXCHANGE. The respiratory exchange was determined by means of an open-circuit apparatus which consisted of a helmet (Benedict, 1930), two blowers, a spirometer, the Fox bag method of sampling the air current (Carpenter and Fox, 1931) and two dry gas meters (Benedict and Ritzman, 1931). Samples of air from the Fox bag were drawn through a calcium-chloride tube into mercury samplers or into a metal pump sampler (Benedict and Ritzman, 1931) and later analyzed by an apparatus (Carpenter, Lee, and Finnerty, 1930) designed for the analysis of chamber air. The ventilation through the apparatus was adjusted so that the carbon-dioxide content was approximately 1 per cent. The gas-analysis apparatus was standardized with outdoor air each day.

The average composition of outdoor air in a series of 76 analyses was 0.031 per cent carbon dioxide and 20.940 per cent oxygen, with standard deviations of 0.0017 per cent for carbon dioxide and 0.0038 per cent for oxygen. Usually all of the samples for the 13 or 14 periods were analyzed at least once on the day of the experiment, and then duplicate analyses were made either on the same day or on the day following, particularly on those samples of which the analyses seemed to be aberrant, or those that were of special importance, as for example, a base-line period, the one having the highest respiratory quotient, and the one at the end of the series. The average of the differences in 74 pairs of analyses was 0.005 per cent for carbon dioxide and 0.008 per cent for oxygen. Occasionally analyses of outdoor air were made at the end of the day's series as an additional control.

Procedure of an experiment. The subject was J. C., 48 years, 72.1 kilos, and 167 cm. He arrived at the Laboratory at about 8:30 a.m., urinated, and then sat quietly in a semi-reclining chair for one-half hour, during which his buccal temperature was taken, and records of his previous meal and other data were obtained. The helmet was then adjusted and the ventilation through the apparatus was started. In the experiments in the spring of 1931, four 15-minute periods were run continuously for the base line for the experiment. In the experiments in the fall of 1931, the ventilation was run for 15 minutes without making any records, and then three 15-minute periods were made, as the previous experience had shown that the first 15-minute period was one of adjustment, and therefore was not of value as a base-line measurement. After the base-line periods were finished, the helmet was removed, and the subject rested for 15 minutes to one-half hour, at the end of which the dose for the experiment was given. The sugar was dissolved in 200 cc. of water at 37°C., which the subject drank in as short a time as possible. The helmet was then adjusted, and ten 15-minute periods were run continuously, the helmet was removed, and the subject urinated.

Urine. The statistics of the urinary elimination for the experiments in this group are given in table 1 in which are shown the duration of the period, the volume per hour, the results of the S. R. Benedict (1908) qualitative test, and the amounts of sugar as found by the S. R. Benedict (1911) quantitative method. The subject on arrival at the Laboratory emptied his bladder and then urine was again collected at the end of the experiment, so that there was no separation of periods before and after the ingestion of sugar. Of the total period of time, about 3 to 3½ hours covers the time after the ingestion of the sugar. The volumes per hour averaged highest in the two groups with 30 and 40 grams of galactose. In the other groups the volume is more irregular, although in general the values tend to be nearly as high as those in the three groups mentioned but the excretion is not so regular. In all of the experiments 200 cc. of water were given.

Consequently if there were no need for a water retention, we would expect an excretion which would be equivalent to the amount of water given plus the normal secretion taking place during the given period of time. In one experiment with 5 grams, 2 experiments with 10 grams and one with 20 grams the volume was below the general average. Whether this is due to a retention of water which accompanies the storage of the sugar in the tissues cannot be stated. The high values with the 30 and 40 grams may be due either to a diuresis accompanying the excretion of sugar or to an increased

TABLE 1

Statistics of urine in galactose experiments with Mr. J. C.

DATE	PERIOD	VOLUME PER HOUR	REDUCING SUGAR	
			Qualita- tive*	Quanti- tative**
1931		cc.		grams
5 grams galactose:				
May 2.....	8:27 a.m.-12:48 p.m.	97	N	
May 4.....	8:18 a.m.-12:48 p.m.	87	N	
May 8.....	8:17 a.m.-12:43 p.m.	35	N	
10 grams galactose:				
April 23.....	8:23 a.m.-12:52 p.m.	77	N	0.7
October 5.....	6:30 a.m.-12:57 p.m.	15	P	0.7
October 7.....	8:30 a.m.-12:52 p.m.	31	P	0.6
20 grams galactose:				
March 18.....	8:38 a.m.- 1:20 p.m.	76	P	
March 23.....	8:25 a.m.-12:49 p.m.	65		0.9
October 9.....	8:27 a.m.-12:53 p.m.	35	P	1.1
30 grams galactose:				
March 19.....	8:33 a.m.-12:53 p.m.	84		2.1
March 24.....	8:35 a.m.-12:50 p.m.	88		2.2
40 grams galactose:				
March 20.....	8:32 a.m.-12:49 p.m.	86		3.6
March 25.....	8:33 a.m.-1:04 p.m.	86		2.5

* N = negative, P = positive.

** Amount of sugar calculated as galactose for the entire period of collection.

excretion of water due to combustion of the sugar. The water due to the combustion of the sugar would amount to 24 grams with 40 grams of galactose.

The excretion of sugar in the urine occurred in all the experiments with 10 grams or over. With the higher amounts of ingested galactose, there were larger amounts of reducing sugars found in the urine, and there is nearly a proportional relationship between the amount of galactose ingested and the amount of sugar in the urine. This subject had a low tolerance for galactose.

The respiratory quotient, combustion of carbohydrates, and heat production after the ingestion of galactose. The respiratory quotients before and after the ingestion of 5 to 40 grams of galactose are given in table 2 for the average of the periods of the base-line hour and for the individual periods follow-

TABLE 2
Respiratory quotients before and after ingestion of galactose (Mr. J. C.)

DATE	BASE LINE	PERIODS AFTER INGESTION OF GALACTOSE									
		1	2	3	4	5	6	7	8	9	10
		0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.
1931											
5 grams galactose:											
May 2.....	0.86	0.85	0.90	0.87	0.83	0.82	0.82	0.81	0.79	0.82	0.80
May 4.....	0.90	0.91	0.92	0.89	0.88	0.87	0.87	0.87	0.86	0.84	0.85
May 8.....	0.83	0.83	0.87	0.90	0.87	0.85	0.83	0.83	0.81	0.82	0.81
Average.....	0.86	0.86	0.90	0.89	0.86	0.85	0.84	0.84	0.82	0.83	0.82
10 grams galactose:											
April 23.....	0.85	0.81	0.83	0.93	0.89	0.84	0.84	0.83	0.83	0.82	0.80
Oct. 5.....	0.80	0.80	0.81	0.82	0.80	0.79	0.80	0.79	0.79	0.80	0.80
Oct. 7.....	0.84	0.84	0.88	0.89	0.88	0.84	0.82	0.83	0.83	0.82	0.83
Average.....	0.83	0.82	0.84	0.88	0.86	0.82	0.82	0.82	0.82	0.81	0.81
20 grams galactose:											
March 18.....	0.84	0.83	0.91	0.98	0.97	0.86	0.86	0.80	0.79	0.80	0.80
March 23.....	0.82	0.82	0.85	0.94	1.01	0.90	0.89	0.85	0.86	0.83	0.84
Oct. 9.....	0.86	0.84	0.88	0.96	0.96	0.89	0.88	0.87	0.84	0.85	0.83
Average.....	0.84	0.83	0.88	0.96	0.98	0.88	0.88	0.84	0.83	0.83	0.82
30 grams galactose:											
March 19.....	0.86	0.86	0.94	0.99	1.01	0.93	0.88	0.86	0.84	0.83	0.81
March 24.....	0.82	0.91	0.97	0.99	0.98	0.90	0.85	0.84	0.82	0.81	0.81
Average.....	0.84	0.89	0.96	0.99	1.00	0.92	0.87	0.85	0.83	0.82	0.81
40 grams galactose:											
March 20.....	0.82	0.81	0.92	0.96	0.99	1.00	0.95	0.90	0.85	0.85	0.83
March 25.....	0.84	0.87	0.97	1.00	0.99	1.02	0.97	0.91	0.85	0.85	0.85
Average.....	0.83	0.84	0.95	0.98	0.99	1.01	0.96	0.91	0.85	0.85	0.84

ing the ingestion of the sugar. The effect of the ingestion of 5 to 40 grams of galactose on the combustion of carbohydrates is shown by the data given by periods in table 3. The changes in heat production caused by the ingestion of varying quantities of galactose are recorded in table 4 for the individual periods. The heat production was calculated from the oxygen

consumption and the respiratory quotient, with an allowance for protein equivalent to a urinary elimination of nitrogen of 0.4 gram per hour. In all three tables, the values were derived from the protocols, which were calculated to one more decimal place than is given in the tables. There

TABLE 3

Carbohydrate metabolism as influenced by the ingestion of galactose (Mr. J. C.)

DATE	BASE LINE*	CHANGES FROM BASE LINE IN PERIODS AFTER INGESTION OF GALACTOSE											Total change**
		1	2	3	4	5	6	7	8	9	10		
		0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.		
1931	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	
5 grams ga- lactose:													
May 2.....	1.83	+0.1	+0.7	+0.1	-0.4	-0.6	-0.4	-0.6	-0.9	-0.6	-0.8	-3.4	
May 4.....	2.39	+0.3	+0.4	-0.1	-0.3	-0.5	-0.5	-0.5	-0.6	-0.9	-0.7	-3.4	
May 8.....	1.23	+0.2	+0.7	+1.0	+0.6	+0.4	+0.2	0.0	-0.1	0.0	-0.1	+3.1	
Average..	1.82	+0.2	+0.6	+0.3	0.0	-0.2	-0.3	-0.3	-0.5	-0.5	-0.5	-1.2	
10 grams ga- lactose:													
April 23....	1.67	-0.6	-0.3	+1.3	+0.6	-0.3	-0.3	-0.3	-0.4	-0.3	-0.6	-1.2	
Oct. 5.....	1.10	0.0	+0.2	+0.4	0.0	-0.1	0.0	-0.1	-0.1	0.0	-0.2	+0.1	
Oct. 7.....	1.57	0.0	+0.6	+0.7	+0.5	-0.1	-0.2	-0.2	-0.2	-0.4	-0.2	+0.3	
Average..	1.45	-0.2	+0.2	+0.8	+0.3	-0.2	-0.2	-0.2	-0.3	-0.2	-0.3	-0.3	
20 grams ga- lactose:													
March 18...	1.61	-0.2	+1.2	+1.9	+1.7	+0.2	+0.2	-0.5	-0.8	-0.5	-0.6	+2.7	
March 23...	1.32	0.0	+0.4	+1.5	+1.9	+1.0	+0.8	+0.3	+0.4	+0.1	+0.1	+6.5	
Oct. 9.....	1.70	-0.3	+0.4	+1.5	+1.3	+0.4	+0.2	0.0	-0.3	-0.2	-0.5	+2.4	
Average..	1.54	-0.2	+0.7	+1.6	+1.6	+0.5	+0.4	-0.1	-0.2	-0.2	-0.3	+3.9	
30 grams ga- lactose:													
March 19...	1.75	+0.1	+1.6	+1.9	+1.7	+1.2	+0.4	+0.3	-0.1	-0.4	-0.6	+6.1	
March 24...	1.37	+1.3	+2.3	+2.2	+2.0	+1.1	+0.4	+0.2	-0.1	-0.2	-0.2	+8.8	
Average..	1.56	+0.7	+2.0	+2.0	+1.9	+1.2	+0.4	+0.2	-0.1	-0.3	-0.4	+7.5	
40 grams ga- lactose:													
March 20...	1.22	+0.1	+1.8	+2.3	+2.5	+2.3	+2.0	+1.2	+0.6	+0.5	+0.2	+13.5	
March 25...	1.56	+0.5	+2.3	+2.2	+2.1	+2.0	+1.7	+1.0	+0.2	+0.1	+0.2	+12.2	
Average..	1.39	+0.3	+2.1	+2.2	+2.3	+2.2	+1.9	+1.1	+0.4	+0.3	+0.2	+12.8	

* For 15 minutes.

** For 2½ hours.

are, therefore, some arithmetical discrepancies between the averages and totals given and those found by calculation from the individual values by periods given in the tables.

TABLE 4
Heat production as influenced by the ingestion of galactose (Mr. J. C.)

DATE	BASE LINE*	CHANGES FROM BASE LINE IN PERIODS AFTER INGESTION OF GALACTOSE											Total change**
		1	2	3	4	5	6	7	8	9	10		
		0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.		
	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.	
5 grams ga- lactose:													
May 2.....	16.6	+0.5	+0.5	+0.1	+0.6	-0.3	0.0	0.0	+0.2	-0.3	+0.1	+1.4	
May 4.....	16.6	+0.4	+0.2	-0.2	-0.6	-0.1	-0.6	-1.0	-0.4	-0.9	-0.3	-3.4	
May 8.....	16.1	+0.2	0.0	-0.3	-0.8	-0.8	-0.4	-0.8	-0.1	-0.7	-0.6	-4.3	
Average..	16.4	+0.3	+0.2	-0.1	-0.3	-0.4	-0.3	-0.6	-0.1	-0.6	-0.3	-2.1	
10 grams ga- lactose:													
April 23....	16.2	-0.5	+0.5	+1.7	+0.1	-0.3	-0.4	-0.5	-1.2	-0.2	+0.2	-0.5	
Oct. 5.....	17.2	+0.3	+0.8	+0.3	-0.2	+0.2	+0.2	-0.1	-0.2	-0.2	-0.7	+0.5	
Oct. 7.....	16.2	+0.2	+1.0	+0.1	-0.6	-0.8	-0.2	0.0	-0.3	-0.5	-0.1	-1.2	
Average..	16.5	0.0	+0.8	+0.7	-0.2	-0.3	-0.1	-0.2	-0.6	-0.3	-0.2	-0.4	
20 grams ga- lactose:													
March 18...	16.6	+0.5	+1.8	+1.0	+0.1	+0.1	-0.2	+0.3	0.0	+0.2	-0.1	+3.7	
March 23...	15.9	+0.3	+0.8	+0.7	+0.4	-0.5	-0.2	-0.7	-0.4	0.0	-0.2	+0.3	
Oct. 9.....	15.5	+0.1	+1.5	+1.0	-0.1	-0.8	-0.9	-1.0	-0.6	-0.7	-1.1	-2.6	
Average..	16.0	+0.3	+1.4	+0.9	+0.2	-0.4	-0.4	-0.5	-0.3	-0.2	-0.5	+0.5	
30 grams ga- lactose:													
March 19...	15.9	+1.0	+2.7	+2.0	+1.4	+1.3	+1.0	+1.1	+0.7	+0.5	+0.7	+12.4	
March 24...	16.3	+1.0	+1.8	+1.4	+0.7	+0.8	+0.2	-0.3	-0.3	-0.2	-0.2	+4.9	
Average..	16.1	+1.0	+2.2	+1.7	+1.1	+1.0	+0.6	+0.4	+0.2	+0.1	+0.3	+8.7	
40 grams ga- lactose:													
March 20...	15.9	+1.5	+2.0	+2.0	+2.4	+1.7	+1.5	+0.6	+0.5	+0.6	+0.4	+13.2	
March 25...	16.1	+1.4	+2.9	+2.3	+1.9	+1.6	+0.4	+0.5	+0.8	-0.2	+0.5	+12.0	
Average..	16.0	+1.4	+2.5	+2.1	+2.2	+1.6	+0.9	+0.6	+0.7	+0.2	+0.4	+12.6	

* For 15 minutes.

** For 2½ hours.

Five grams of galactose. After the ingestion of 5 grams of galactose there was a rise of 0.02 to 0.05 in the respiratory quotient in the second or third period, even though two of the average base-line quotients were 0.90 and 0.86. The average maximum rise in the 3 experiments was 0.04 in the second period. From there on there was a gradual fall until the end of the series when the quotient was 0.04 below the average base line. There was an increase in the combustion of carbohydrates in the first three periods on the average. The experiment of May 8 differs from the others in that the increase in the combustion of carbohydrate was much larger than in the other two experiments and persists through period 6 (75 to 90 min.). This experiment had the lowest combustion of carbohydrates in the base-line periods, which would lead one to expect a low increase in carbohydrate combustion. There was an increase in the heat production in the first two periods. After the second period, there was a fall in the heat production with three exceptions, periods 3, 4 and 8 of May 2.

Ten grams of galactose. The ingestion of 10 grams of galactose was followed by a maximum rise of 0.08 in the respiratory quotient in the third quarter-hour on April 23 and by an average maximum rise in the 3 experiments of 0.05 in the third period after ingestion. There was a sharp fall in the average quotient from the third to the fifth period after ingestion. The experiment of October 5 differed from the other two in that there was a rise of only 0.02 in the quotient. This experiment was the first of the series in the fall of 1931. The average of 3 observations of buccal temperature on October 5 was 98.7°F., whereas the maximum in any other experiment was 98.1 on April 8. The subject's base-line heat production on October 5 was 17.2 calories per 15 minutes in comparison with a range of from 15.5 to 16.6 calories in all other experiments in this series. He was, therefore, on a distinctly higher plane of metabolism in the experiment of October 5 than in the other two experiments in the group with 10 grams of galactose. There was a definite rise in combustion of carbohydrates in the second and third quarter-hours on October 5 and 7 and in the third and fourth quarter-hours on April 23. After period 4, the values were either nearly those of the pre-ingestion periods or fell below those values. There was a slightly greater increase in heat production than after the ingestion of 5 grams of galactose. The maximum was 1.7 calories in period 3 of April 23, equivalent to 10 per cent of the base line. After the third period, the changes were mostly negative, although not so large as a whole as those in the same periods after the ingestion of 5 grams of galactose. Thus, there was not only a slightly greater increase in the first part of the experiments, but also a smaller fall in the latter part of the experiments in this group than with the preceding group.

Twenty grams of galactose. The ingestion of 20 grams of galactose was followed by a maximum rise of 0.19 in the respiratory quotient in period 4

(45 to 60 min.) on March 23, and by an average maximum rise of 0.14 in the 3 experiments in this same period. There was a rise in the combustion of carbohydrates in periods 2 to 6, inclusive, in all of the experiments and in all of the periods on March 23. The average maximum rise was 1.6 grams in periods 3 and 4, that is, twice as much as with 10 grams of galactose. The last three periods of the group showed about the same fall in combustion of carbohydrates as in the same periods with 10 grams of galactose. There were marked increases in heat production in the second and third periods after ingestion. The maximum rise was 1.8 calories in period 2 of March 18, equivalent to 11 per cent of the base line. The average heat increment in period 2 was 1.4 calories, equivalent to 9 per cent of the average base line.

Thirty grams of galactose. The ingestion of 30 grams of galactose was followed by an average maximum rise of 0.16 in the respiratory quotient in the fourth period (45 to 60 min. after ingestion) of the two experiments, and a rapid fall to the base-line value in the next 3 periods. There was a rise in the combustion of carbohydrates in periods 1 to 7 (90 to 105 min.) in both experiments. The maximum increase in carbohydrate combustion was 2.3 grams in the second quarter-hour on March 24. The maximum rise in heat production was 2.7 calories in the second period (15 to 30 min.) on March 19, equivalent to 17 per cent of the base line. The average rise in this period was 2.2 calories, equivalent to 14 per cent of the base line. In all of the following periods the heat production was, on the average, above the base line, with the minimum average value of +0.1 calorie in period 9.

Forty grams of galactose. The ingestion of 40 grams of galactose was followed by a maximum rise in the respiratory quotient of 0.18 in period 5 (60 to 75 min.) in both experiments, with a rapid fall to a value just above or at the base-line level for the last 3 periods of the experiments. This group was the only group in which the respiratory quotient remained above the base line or pre-ingestion level for two and one-half hours. The greatest rise in combustion of carbohydrates was 2.5 grams in period 4 on March 20 and the greatest average rise was 2.3 grams in period 4. In both experiments the combustion of carbohydrates was greater than the pre-ingestion level throughout all of the periods. There was a rise in heat production in all of the periods in the two and one-half hours of the experiments except period 9 on March 25. The maximum rise in any period was 2.9 calories in period 2 on March 25, equivalent to 18 per cent of the base line.

The ingestion of galactose in quantities varying from 5 to 40 grams was followed by a definite rise in the respiratory quotient. The rise was not directly proportional to the quantity ingested, although each increasingly larger quantity resulted in a successively larger increase. The greatest difference in the rises in quotient between two quantities was 0.09 between

that after 10 grams and that after 20 grams. The period of maximum rise tended to occur about 15 minutes later in each successive group, beginning with period 2 in 5-gram experiments and ending with period 5 in the 40-gram experiments. This differs from the experiments with glucose (Carpenter and Fox, 1930a) in which the maximum quotient appeared 45 to 75 minutes after the ingestion and from the experiments with fructose (Carpenter and Fox, 1930b) in which the maximum quotient was in period 3 (30 to 45 min.) irrespective of the quantity.

The effect on the respiratory quotient of the ingestion of galactose may be estimated in another way, that is, by comparing the average respiratory quotient for two and one-half hours after ingestion with the average base-line quotient. The average of the differences between the average quotient after the base-line hour and that during the base-line hour for each of the different groups is as follows: 5 grams of galactose, -0.006 ; 10 grams of galactose, -0.001 ; 20 grams of galactose, $+0.031$; 30 grams of galactose, $+0.051$; and 40 grams of galactose, $+0.084$. Thus there was an increase in the effect of the galactose upon the quotient for two and one-half hours with each succeeding increasing quantity. The average increases per 10-gram steps are more uniform than the maximum rises of quotients over the base line.

The summation of the average increases in combustion of carbohydrates in the successive periods (in which there was an increase) gives the following results for the different groups with galactose: 5 grams, $+1.1$; 10 grams, $+1.3$; 20 grams, $+4.8$; 30 grams, $+8.4$; and 40 grams, $+12.8$ grams of carbohydrates. The differences between the successive groups are $+0.2$, $+3.5$, $+3.6$, and $+4.4$. Thus the largest increase, 4.4 grams, per 10-gram increase in amount given is that between 30 and 40 grams. However, having regard to the relatively small number of experiments in each group, we can tentatively state that for each 10-gram increase in amount of galactose ingested, the additional increase in apparent combustion of carbohydrates was nearly of the same order of magnitude.

When the increases in carbohydrate combustion are expressed as percentages of the amounts of galactose ingested, the values are: 5 grams, 22; 10 grams, 13; 20 grams, 24; 30 grams, 28; and 40 grams, 32 per cent. Thus, with the exception of the 10-gram group, the increase in combustion of carbohydrates in terms of the amount ingested rises slightly and regularly with each successively larger amount of galactose ingested. These values are slightly larger and more regular than those found by Carpenter and Fox (1930a) with glucose and are about the same as those found by the same authors with fructose (Carpenter and Fox, 1930b), but also somewhat more regular than with fructose. The greater regularity may have been due to the use of the helmet, or to the fact that the experiments were

made with the subject in the sitting position, or to a combination of both causes.

The summation of increases in the heat production of the several groups gives the following results: 5 grams galactose, +0.5; 10 grams, +1.5; 20 grams, +2.8; 30 grams, +8.7; and 40 grams, +12.6 calories. Not all of the probable increase in heat production was measured in the 30-gram and 40-gram experiments, especially in the latter group, as the last periods still resulted in increases above the base line. The greatest difference per 10 grams in the increase in the heat production is that between 20 and 30 grams, that is, 5.9 calories. It is probable that the true difference is larger than this, because of the fact that not all of the increase was measured as the experiments did not last long enough.

The stimulating influence on the heat production in relation to the heat of combustion of the ingested galactose, that is, the specific dynamic action, is as follows for the different amounts of sugar: 5 grams, 3; 10 grams, 4; 20 grams, 4; 30 grams, 8; and 40 grams, 8 per cent. Below 20 grams, the specific dynamic action is thus 3 to 4 per cent and above 20 grams rises more rapidly than the increases in amounts given.

The influence of galactose on the heat production was somewhat like that of glucose (Carpenter and Fox, 1930a) in that with the smaller quantities the increases were small, but with the larger quantities, the increases in heat production rose more proportionately than the amounts ingested.

SUMMARY

The effects on the respiratory quotient and the total metabolism of the ingestion of 5 to 40 grams of galactose were determined with a human subject by means of an open-circuit respiration apparatus and a helmet. The base line for the day was measured in three to four 15-minute periods, and ten 15-minute periods were run after the sugar was taken.

The subject had a low tolerance for this sugar as the total reducing substances in urine for approximately 3 hours varied from 0.7 gram after the ingestion of 5 grams to 3.1 grams after the ingestion of 40 grams of galactose.

All amounts of galactose caused a rise in the respiratory quotient, the maximum rise in a period varying from 0.04 with 5 grams to 0.17 with 40 grams. This sugar resembles somewhat fructose in the effects on the quotient, although it differs from both fructose and glucose in that there was a fall to below the pre-ingestion level during the latter part of the two and one-half hours after ingestion.

The rise in apparent carbohydrate combustion varied from 1.1 to 12.8 grams, which represented from 13 to 32 per cent of the amount ingested.

The maximum increase in heat production over the base-line level in a 15-minute period varied from 10 per cent with 10 grams to 18 per cent with 40 grams.

The summation of increases in heat production in successive periods varied from 0.6 calorie with 5 grams to 12.6 calories with 40 grams and the specific dynamic action (the increase in heat production in comparison to the heat of combustion of the sugar) varied from 3 to 8 per cent.

Galactose resembles fructose in its effects on the respiratory quotient and carbohydrate combustion and resembles glucose in its effects on the heat production.

We are indebted to Dr. Allan Winter Rowe of the Evans Memorial Hospital of Boston for providing us with the galactose.

BIBLIOGRAPHY

- BENEDICT, F. G. 1930. *New England Journ. Med.*, cciii, 150.
BENEDICT, S. R. 1908. *Journ. Biol. Chem.*, v, 485.
1911. *Journ. Biol. Chem.*, ix, 57.
BENEDICT, F. G. AND E. G. RITZMAN. 1931. *Wissenschaftliches Arch. f. Landwirtschaft, Abt. B*, v, 1.
CARPENTER, T. M. AND E. L. FOX. 1930a. *Journ. Nutr.*, ii, 375.
1930b. *Journ. Nutr.*, ii, 389.
1931. *Arbeitsphysiol.*, iv, 527.
CARPENTER, T. M., R. C. LEE AND A. E. FINNERTY. 1930. *Wissenschaftliches Arch. f. Landwirtschaft, Abt. B*, iv, 1.
CATHCART, E. P. AND J. MARKOWITZ. 1927. *Journ. Physiol.*, lxiii, 309.
DEUEL, H. J., JR. 1927. *Journ. Biol. Chem.*, lxxv, 367.
LUSK, G. 1915. *Journ. Biol. Chem.*, xx, 555.
WIERZUCHOWSKI, M. 1931. *Biochem. Zeitschr.*, ccxxx, 187.

A COMPARISON OF THE RESPIRATORY EXCHANGE OF MEN AND WOMEN AS AFFECTED BY THE INGESTION OF GALACTOSE

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A difference in metabolism between the two sexes has been found in a number of investigations. The basal metabolism has been observed to be higher in the male with the young human (Benedict and Talbot, 1921); with the adult human (Benedict and Emmes, 1915); with the fowl (Mitchell and Haines, 1927, Mitchell, Card, and Haines, 1927); with the rat (Benedict and MacLeod, 1929); and with the ring dove (Riddle, Christman, and Benedict, 1930). Very recently the last named authors have demonstrated that the variation in heat production according to temperature varies with the ring doves according to sex. Greisheimer (1931) found a materially lower percentage of glycogen in the liver of female rats than that in the liver of male rats after 48 hours of fasting. Deuel and Gulick (1932) noted that a greater ketosis developed in the human female than in the male during fasting. Rowe (1924) found a difference between the sexes in the tolerance for galactose in the human adult in that the average amount of galactose which brought about a S. R. Benedict (1908) qualitative test in the 4-hour urine collected after ingestion was 30 grams with the males, whereas with the females it was 40 grams. Harding and Moberley (1930) confirmed Rowe so far as the qualitative test was concerned, but quantitatively the women excreted more galactose than the men. They offered as a possible explanation the difference in response to the diuretic action of water rather than a real sex difference in the metabolism of galactose. The individual variation was large, so that they stated no definite conclusion could be substantiated. Roe and Schwartzmann (1932) gave 1 gram of galactose per kilo to 5 normal men and 5 normal women and found no evidence for a higher tolerance by women than by men.

While our other studies on the respiratory exchange after the ingestion of galactose (Carpenter and Lee, 1932) were going on, opportunity was offered us by Dr. Allan Winter Rowe to make some observations on members of his staff on the effect of galactose on the respiratory exchange. The apparatus used and the procedure of an experiment were the same as in

the preceding investigation. The average of 68 analyses of outdoor air was 0.031 per cent for carbon dioxide and 20.940 per cent for oxygen, with standard deviations of 0.0014 and 0.0036 per cent, respectively. The average of the differences in 126 pairs of analyses of the experimental samples was 0.004 per cent for carbon dioxide and 0.007 per cent for oxygen. The observations were carried out at the Nutrition Laboratory. Although some of the subjects had had experience with respiration apparatus, none of them had ever worn the helmet (Benedict, 1930) as a breathing appliance. No previous training was given them in the use of the helmet, so that the first experiment recorded with each subject was the first experience.

Statistics of subjects. The statistics of the 5 men and 6 women who acted as subjects are given in table 1. They were all presumably normal.

TABLE 1
Statistics of subjects

SUBJECT	AGE		HEIGHT	WEIGHT WITH CLOTHES
	<i>yr.</i>	<i>mos.</i>	<i>cm.</i>	<i>kilos</i>
M 1	51	8	165	52.4
M 2	28	9	173	74.9
M 3	35	9	173	86.7
M 4	22	1	186	78.3
M 5	48	0	167	72.1
F 1	21	0	163	51.1
F 2	28	10	164	62.1
F 3	27	5	165	65.5
F 4	23	10	155	51.8
F 5	23	9	170	72.7
F 6	23	10	159	50.7

M-1 is one of the authors (T. M. C.) and M-5 is J. C., the subject of the preceding investigation. The results with M-5 are not included in the averages in tables 3 to 8 because he was the subject in more than one experiment. All weights and ages are the averages for the two experiments in which the subjects were used. F-2 had a weight of 60.1 kilos in 1930 and 64.1 kilos in 1931.

Statistics of urine. The statistics of the urinary elimination in these experiments are given in table 2, namely, the subject, date, period covered, volume per hour, and the results of the S. R. Benedict (1908) qualitative test and the quantitative determination by the S. R. Benedict (1911) method. Unfortunately at the time that the experiments were made, the importance of a careful and rigid examination of the urine for sugar was not recognized, as presumably all of the subjects were normal. Consequently the data are fragmentary and incomplete. Two of the males, M-1

and M-5, gave a positive test with 20 grams; the other three were negative. With 30 grams two, M-2 and M-3, were negative, the same as with 20 grams. M-4 and M-5 were positive. Of the females, four were negative

TABLE 2
Statistics of urine in galactose experiments with men and women

AMOUNT INGESTED	SUBJECT	DATE	PERIOD	VOLUME PER HOUR	REDUCING SUGAR	
					Qualita- tive†	Quantita- tive‡
Men						
grams		1930		cc.		grams
20	M 1	May 26	8:11 a.m.-12:45 p.m.	24	P	0.4
	M 2	July 10	8:20 a.m.- 1:00 p.m.		N	
	M 3	July 11	8:40 a.m.- 1:10 p.m.		N	
	M 4	July 14	8:45 a.m.- 1:17 p.m.	110	N	
	M 5*			59	P	1.0
30	M 2	July 17	8:39 a.m.- 1:03 p.m.	86	N	
	M 3	July 18	8:37 a.m.- 1:08 p.m.	11	N	
	M 4	July 21	8:00 a.m.- 1:18 p.m.	22	P	0.8
	M 5**			86		2.1
Women						
20	F 1	July 7	1:15 p.m.		N	
	F 2	July 9	8:40 a.m.- 1:05 p.m.		N	
	F 3	July 16	8:20 a.m.-12:55 p.m.	9	N	
		1931				
	F 4	April 14	8:32 a.m.-12:51 p.m.	137	N	
	F 5	April 24	8:33 a.m.- 1:00 p.m.	182	N (?)	
30	F 6	May 7	8:53 a.m.- 1:32 p.m.	63	P	0.6
		1930				
	F 1	July 29	1:10 p.m.		N	
		1931				
	F 2	May 27	8:02 a.m.-12:45 p.m.	111	P	0.5
	F 4	April 28	8:27 a.m.- 1:00 p.m.	125	P	0.8
	F 6	May 14	9:00 a.m.- 1:18 p.m.	24	P	0.8

* Average of 3 experiments. See preceding article, page 635.

** Average of 2 experiments.

† N = negative, P = positive, Benedict qualitative test.

‡ Amount in total urine voided.

with 20 grams, in one, F-5, the test was uncertain, and F-6 was positive. With 30 grams only one, F-1, was negative out of the four whose urines were tested. The later discussion will show that with F-5 and F-6 the respiratory quotient rose less than with the other females. The data

on the volume are too fragmentary and too limited to discover any relationship between the presence or absence of a qualitative test and the diuresis.

THE RESPIRATORY EXCHANGE OF MEN AND WOMEN AFTER THE INGESTION OF GALACTOSE. *Respiratory quotient.* The respiratory quotients for the base-line period and for the ten 15-minute periods following the ingestion

TABLE 3

Respiratory quotients of men and women before and after the ingestion of 20 grams galactose

SUBJECT	DATE	BASE LINE*	PERIODS AFTER INGESTION OF GALACTOSE										
			1	2	3	4	5	6	7	8	9	10	
			0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.	
Males:		1930											
M 1.....	May 26	0.81	0.82	0.87	0.94	0.92	0.91	0.84	0.81	0.81	0.80	0.79	
M 2.....	July 10	0.78	0.77	0.78	0.85	0.83	0.83	0.82	0.78	0.80	0.80	0.80	
M 3.....	July 11	0.83	0.82	0.94	0.97	0.85	0.81	0.82	0.81	0.81	0.82	0.81	
M 4.....	July 14	0.80	0.78	0.80	0.89	0.93	0.93	0.81	0.77	0.79	0.80		
Average change from base line...		0.80	-0.01	+0.04	+0.11	+0.08	+0.07	+0.02	-0.01	0.00	0.00	-0.01	
M 5**.....		0.84	-0.01	+0.04	+0.12	+0.14	+0.04	+0.04	0.00	-0.01	-0.01	-0.02	
Females:		1930											
F 1.....	July 7	0.81	0.80	0.86	0.94	0.86	0.81	0.86	0.87	0.82			
F 2.....	July 9	0.78	0.78	0.83	0.92	1.01	0.82	0.81	0.77	0.76	0.77		
F 3.....	July 16	0.80	0.79	0.89	1.01	0.91	0.88	0.80	0.81	0.81	0.79	0.81	
		1931											
F 4.....	April 14	0.78	0.77	0.84	0.91	0.86	0.84	0.83	0.82	0.80	0.79	0.79	
F 5.....	April 24	0.82	0.81	0.84	0.87	0.84	0.85	0.82	0.81	0.84	0.81	0.81	
F 6.....	May 7	0.80	0.76	0.78	0.82	0.85	0.83	0.82	0.79	0.81	0.80	0.80	
Average change from base line...		0.80	-0.01	+0.04	+0.11	+0.09	+0.04	+0.02	+0.01	+0.01	-0.01	0.00	

* For 15 minutes.

** Average of 3 experiments. See preceding article, page 635.

of 20 grams of galactose are given in table 3 and for the same periods before and after the ingestion of 30 grams of galactose in table 4.¹ With 20 grams of galactose there was but little change in both sexes in the first period after the ingestion. The average maximum rise above the base-line values was of the same order, namely, 0.11 in the third period (30 to 45 min.) after ingestion. With subject M-5, who was used in the previous investigation, the maximum increase in the respiratory quotient was slightly greater than

¹ The results in tables 3 to 8 are taken from the original protocols which were calculated to the second decimal place. There are some apparent errors in the tables, therefore, when an attempt is made to check the averages or the totals.

with either of the other two groups and occurred in a later period. The fall to the base line after the ingestion took place with both groups in the 7th period (90 to 105 min.) and from there on there is no significant change. There were wide differences in the individual maximum increases in the respiratory quotient in both groups. In the male group, M-2 showed the lowest maximum increase of 0.07, whereas M-1 and M-3 had maximum increases of 0.13 and 0.14 in the third period after ingestion and M-4 had +0.13 in the fourth period after ingestion. None of these are as high as with M-5. With the women the maximum increases were with F-2 in

TABLE 4

Respiratory quotients of men and women before and after the ingestion of 30 grams galactose

SUBJECT	DATE	BASE LINE*	PERIODS AFTER INGESTION OF GALACTOSE									
			1	2	3	4	5	6	7	8	9	10
			0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.
Males:	1930											
M 2.....	July 17	0.82	0.80	0.87	0.96	0.96	1.02	0.94	0.91	0.84	0.84	0.84
M 3.....	July 18	0.83	0.85	0.98	0.99	0.95	0.87	0.84	0.84	0.83	0.82	0.80
M 4.....	July 21	0.79	0.82	0.92	0.97	0.93	0.84	0.83	0.82	0.79	0.81	0.82
Average change from base line....		0.82	+0.01	+0.11	+0.16	+0.13	+0.10	+0.05	+0.04	0.00	+0.01	0.00
M 5**.....		0.84	+0.05	+0.12	+0.15	+0.16	+0.08	+0.03	+0.01	-0.01	-0.02	-0.03
Females:	1930											
F 1.....	July 29	0.88	0.91	0.95	1.07	1.01	0.94	0.90	0.89	0.91	0.93	0.89
F 3.....	July 30	0.84	0.82	0.92	1.03	1.04	1.01	0.89	0.86	0.85	0.86	
	1931											
F 2.....	May 27	0.81	0.81	0.90	0.94	1.00	0.95	0.86	0.82	0.80	0.79	0.79
F 4.....	April 28	0.80	0.76	0.82	0.93	1.01	0.97	0.86	0.80	0.82	0.83	0.79
F 6.....	May 14	0.79	0.77	0.80	0.87	0.91	0.92	0.91	0.87	0.85	0.80	0.77
Average change from base line....		0.82	-0.01	+0.06	+0.15	+0.17	+0.14	+0.06	+0.02	+0.02	+0.02	-0.01

* For 15 minutes.

** Average of 2 experiments. See preceding article, page 635.

the fourth period (45 to 60 min.) after ingestion, namely, 0.23, and with F-3 in the period preceding, namely, 0.21. The lowest maximum increases were with F-5 and F-6, 0.05 in the third and fourth periods, respectively. There is thus a greater variation in the maximum increases in the respiratory quotient with the women than with the men. The cause for the small increase with M-2 is not known. He had been nearly 24 hours without food whereas the others had abstained from food for only about 12 to 15 hours. There were also individual differences with regard to the rate at which the quotient returned to the base line after ingestion. For example, with M-3 the return occurred in the fifth period after ingestion and from then on the values were below the base line, whereas with M-2 there were

both increases and decreases for the entire experiment. Similarly, with F-1 there was not a uniform decline to base line, but rather a variation, the quotient returning to the base line in the fifth period after ingestion and increasing 0.06 in the seventh period. The quotients of F-2 and F-5 returned to base line in the seventh period, but there were some rises later.

In the series with 30 grams of galactose there were only 3 men on whom single experiments were made. With M-5, two experiments were made, the details of which were reported in the preceding publication. The rise with the males came earlier than with the females. For example, in the second period (15 to 30 min.) after ingestion the rise for the 3 males was 0.11 and for M-5, 0.12, whereas with the 5 females it was only 0.06. The maximum rise reached by the males was 0.16 whereas it was 0.17 with the females. The females continued at nearly the same level in the third, fourth, and fifth periods, whereas the males showed a tendency to fall from the high level immediately after the third period. M-5, to a certain extent, resembled the females. The three males showed individual maximum rises varying from 0.20 with M-2 in period 5 to 0.16 with M-3 period 3 after ingestion. With the five women the variations in the individual maximum rises were from 0.21 to 0.13. Four out of five showed maximum rises of 0.19 or over. F-6, who showed one of the smallest rises with 20 grams, also showed the smallest rise here, namely, 0.13.

In general it would seem that the response of the women, so far as the respiratory quotient is concerned, to the ingestion of 30 grams of galactose was slightly more marked in the majority of cases than with the males and the return to the base line was slightly less and slower than that of the males with the exception of M-5. There is thus not a marked difference, on the average, in the rise of the respiratory quotient after 20 and 30 grams with the two sexes, but the variations in the individual rises are slightly greater with the females than with the males.

Relation between maximum rise in respiratory quotient and body weight. An arrangement of the individual maximum rises in respiratory quotients in the order of the increasing body weights of the subjects shows no relation between body weight and increase in quotient.

Relation between maximum rise in respiratory quotient and sugar in urine. There is but little evidence of any relationship between the maximum rise of the respiratory quotient and the amount or appearance of sugar in the urine as indicated by the Benedict qualitative or quantitative determination. In the 20-gram experiments with men the maximum rise in quotient was with M-5 and M-3, one of whom (M-5) showed sugar in the urine in two experiments, the other none. Similarly, the smallest rise was accompanied by no sugar in the urine. The 30-gram experiments with the males all uniformly gave a large maximum rise in the respiratory quotient (range 0.04) with no sugar or with over 3 grams of sugar in the urine. In the 20-

gram experiments with the women the range in the maximum rise was wide (0.18) but only one (F-6) out of 6 showed any sugar in the urine. She also had nearly the smallest rise in respiratory quotient. With the 30-gram experiments with the females, 3 out of 5 had sugar in the urine, but these 3 included the greatest and smallest rise in the respiratory quotient. The only indication of any relationship between the two is with F-6, who had sugar in the urine in both series and also had the smallest or next to the smallest rise in the respiratory quotient.

TABLE 5

Changes from base line of carbohydrate metabolized with men and women after the ingestion of 20 grams galactose

SUBJECT	DATE	BASE LINE*	PERIODS AFTER INGESTION OF GALACTOSE											Total change**
			1	2	3	4	5	6	7	8	9	10		
			0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.		
		gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	
Males:			1930											
M 1.....	May 26	1.02	+0.1	+0.7	+1.4	+1.1	+1.0	+0.3	0.0	0.0	-0.1	-0.2	+4.1	
M 2.....	July 10	1.04	-0.2	0.0	+1.0	+0.7	+0.7	+0.6	0.0	+0.2	+0.2	+0.2	+2.5	
M 3.....	July 11	1.96	-0.1	+2.1	+2.5	+0.2	-0.2	-0.1	-0.3	-0.3	-0.1	-0.3	+4.0	
M 4.....	July 14	1.54	-0.4	+0.1	+1.6	+2.5	+2.2	+0.1	-0.5	-0.1	+0.1		+4.8	
Average.....		1.39	-0.1	+0.7	+1.6	+1.1	+0.9	+0.2	-0.2	-0.1	0.0	-0.1	+3.9	
M 5†.....		1.54	-0.2	+0.7	+1.6	+1.6	+0.5	+0.4	-0.1	-0.2	-0.2	-0.3	+4.3	
Females:			1930											
F 1.....	July 7	1.14	0.0	+0.7	+1.6	+0.6	+0.1	+0.6	+0.7	+0.1			+4.2	
F 2.....	July 9	0.79	+0.1	+0.7	+1.7	+2.4	+0.4	+0.3	-0.1	-0.2	-0.1		+5.3	
F 3.....	July 16	1.05	0.0	+1.2	+2.6	+1.4	+0.9	0.0	+0.1	+0.1	-0.1	+0.2	+6.3	
			1931											
F 4.....	April 14	0.86	-0.1	+0.8	+1.5	+0.8	+0.6	+0.5	+0.4	+0.2	0.0	+0.1	+4.3	
F 5.....	April 24	1.49	+0.1	+0.5	+0.9	+0.4	+0.4	+0.1	0.0	+0.5	-0.1	-0.1	+2.7	
F 6.....	May 7	0.92	-0.3	-0.1	+0.3	+0.6	+0.3	+0.2	-0.1	+0.1	0.0	0.0	+1.4	
Average.....		1.04	0.0	+0.6	+1.4	+1.0	+0.5	+0.3	+0.2	+0.1	-0.1	0.0	+4.0	

* For 15 minutes.

** Total change from base line computed from the metabolism for the entire experiment by means of the total oxygen absorption and the average respiratory quotient.

† Average of 3 experiments. See preceding article, page 635.

Carbohydrate combustion after the ingestion of galactose. The changes in the combustion of carbohydrates during ten 15-minute periods following the ingestion of 20 grams of galactose are shown in table 5 for 5 males and 6 females. The changes by periods were calculated on the assumption that the changes in respiratory quotient were significant and without reference to the participation of protein in the metabolism. The first of these assumptions may not be warranted entirely because part of the rises in respiratory quotient may be due to the formation of acids in the intermedi-

ary metabolism that drive off carbon dioxide with a subsequent retention to compensate for the extra carbon dioxide given off. For comparative purposes between men and women it is believed that the calculated changes serve the purpose. With reference to the second assumption, as long as the protein metabolism was not definitely known, it does not seem worth while to assume an arbitrary value for the subjects. The omission of taking into account the protein results in values given in these tables which are slightly too high.

Both groups of subjects in the 20-gram series showed a definite rise in the carbohydrate combustion in the second period (15 to 30 min.) with a maximum in the third period after ingestion. The greatest rise in any individual period with males was 2.5 grams with M-3 on July 11 in the third period and M-4, fourth period, July 14. Similarly with the females the maximum rise was 2.6 with F-3 on July 16. There is a greater uniformity in the maximum rises with the males than with the females, the range in the latter being from 0.6 with F-6 to 2.6 with F-3. The carbohydrate combustion fell off with the males to the base line or below in 90 minutes after ingestion, whereas with the females it was 120 minutes after ingestion on the average. Subject M-5, with whom 3 experiments were run in the previous investigation, showed about the same order of changes as the other males with a more marked depression after a return to the base line than in the remainder of the group. The total change in carbohydrate combustion for a period of two and one-half hours is given in the last column of the table and shows a uniform rise with the males with the exception of M-2. The female average was practically the same as that of the males, but there was a greater range in the values, that is, from 1.4 to 6.3 grams. F-5 and F-6 gave decidedly lower total increases in carbohydrate combustion than the other four subjects. Excluding these two, the average of the other four would be slightly higher than the general average for the males.

In table 6 are shown the changes in calculated carbohydrate combustion for the experiments with 30 grams. The males gave a greater increase in the carbohydrate combustion practically all through the periods, beginning with the second period (15 to 30 min.) after ingestion, and this rise was shown by all three of the subjects. M-5 had also similarly a rise in the same periods and nearly as marked as the other 3 males. The maximum rise in any single period was 3.4 with M-4 on July 21. With the females marked changes in increases in carbohydrate combustion are shown in periods 3, 4, 5, during the time of 30 to 75 minutes after ingestion, but the increases were not so great on the whole, nor in any single period, as with the males. F-3 is the only subject whose increase in carbohydrate combustion approaches that of the males. Part of the greater increase with the males must be ascribed to the increase in heat production accompanying

the ingestion of 30 grams of galactose. This is shown in table 8. The total increases in carbohydrate combustion for a period of two and one-half hours are shown in the last column of table 6. The 3 males showed a nearly uniform rise, on the average, 10.5 grams of carbohydrate. Subject M-5 did not have quite so large an increase, only 7.9 grams. Four out of the 5 females had a total increase less than the lowest of the 3 males and the average was 7.8 with a maximum value of 9.8 in the series. There was no apparent decrease in carbohydrate combustion in the latter periods of

TABLE 6

Changes from base line of carbohydrate metabolized with men and women after the ingestion of 30 grams galactose

SUBJECT	DATE	BASE LINE*	PERIODS AFTER INGESTION OF GALACTOSE										Total change**
			1	2	3	4	5	6	7	8	9	10	
			0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.	
		gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
Males:	1930												
M 2.....	July 17	1.46	-0.2	+0.8	+2.1	+2.1	+2.4	+1.6	+1.1	+0.3	+0.3	+0.3	+11.2
M 3.....	July 18	1.94	+0.6	+2.8	+3.0	+2.2	+0.8	+0.2	+0.1	0.0	-0.1	-0.4	+9.4
M 4.....	July 21	1.32	+0.6	+2.4	+3.4	+2.5	+0.8	+0.8	+0.5	0.0	+0.4	+0.6	+10.9
Average.....		1.57	+0.3	+2.0	+2.8	+2.2	+1.3	+0.9	+0.6	+0.1	+0.2	+0.2	+10.5
M 5†.....		1.56	+0.7	+2.0	+2.0	+1.9	+1.2	+0.4	+0.2	-0.1	-0.3	-0.4	+7.9
Females:	1930												
F 1.....	July 29	1.73	+0.5	+0.9	+1.5	+1.7	+0.8	+0.4	+0.2	+0.4	+0.6	+0.2	+8.0
F 3.....	July 30	1.51	-0.1	+1.2	+2.3	+2.3	+2.1	+0.7	+0.4	+0.1	+0.2		+9.8
	1931												
F 2.....	May 27	1.23	+0.2	+1.3	+1.7	+2.6	+1.8	+0.6	+0.1	-0.1	-0.3	-0.3	+7.7
F 4.....	April 28	1.02	-0.4	+0.4	+1.5	+2.2	+1.7	+0.6	-0.1	+0.2	+0.4	-0.1	+6.7
F 6.....	May 14	0.89	-0.2	+0.1	+1.0	+1.4	+1.4	+1.3	+0.9	+0.6	+0.1	-0.2	+6.7
Average.....		1.28	0.0	+0.8	+1.6	+2.0	+1.6	+0.7	+0.3	+0.2	+0.2	-0.1	+7.8

* For 15 minutes.

** Total change from base line computed from the metabolism for the entire experiment by means of the total oxygen absorption and the average respiratory quotient.

† Average of 2 experiments. See preceding article, page 635.

the experiments with the males to compensate for the greater increases in the earlier periods with the males as compared with the females. It is apparent that the two quantities of galactose reacted differently with the two sexes, that is, there was a tendency with the females to have larger increases in the carbohydrate combustion with 20 grams, whereas, on the contrary, with 30 grams, the males had the larger increase in carbohydrate combustion.

Heat production after the ingestion of galactose. The heat production for the base-line period and for the changes in heat production in the ten 15-

minute periods following the ingestion of 20 grams of galactose is shown in table 7 for the males and females. The first three periods in both groups gave an increase in heat production, the most marked being in the second period (15 to 30 min.) after ingestion. There is a striking difference in the changes in the heat production in the first period with the females as compared with the males. Without exception the females reacted with a marked increase in the heat production in this period, whereas only one of

TABLE 7

Changes from base line in the heat production of men and women after the ingestion of 20 grams galactose

SUBJECT	DATE	BASE LINE*	PERIODS AFTER INGESTION OF GALACTOSE										Total change**
			1	2	3	4	5	6	7	8	9	10	
			0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.	
Males:	1930	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.
M 1.....	May 26	12.2	+0.2	+0.8	+0.7	+0.1	-0.1	-0.3	-0.4	0.0	+0.4	+0.3	+1.7
M 2.....	July 10	17.4	-0.1	+0.5	+0.5	-0.4	-0.2	0.0	-0.3	-1.1	-0.9	-1.1	-3.4
M 3.....	July 11	19.7	+1.1	+1.9	+1.4	-1.4	+0.8	+1.2	+0.3	+0.6	+0.6	0.0	+6.8
M 4.....	July 14	20.4	-0.5	+1.2	+0.6	+1.9	+0.2	-1.2	+0.4	+0.6	+0.7		+3.8
Average.....		17.4	+0.2	+1.1	+0.8	+0.1	+0.2	-0.1	0.0	0.0	+0.2	-0.2	+2.2
M 5†.....		16.0	+0.3	+1.4	+0.9	+0.2	-0.4	-0.4	-0.5	-0.3	-0.2	-0.5	+0.6
Females:	1930												
F 1.....	July 7	13.6	+1.6	+1.0	+1.1	0.0	+1.4	+0.6	+0.3	+0.1			+5.9
F 2.....	July 9	13.3	+1.1	+1.3	+1.3	+0.3	-0.2	-0.1	+0.3	+0.5	0.0		+4.6
F 3.....	July 16	13.9	+1.8	+1.1	+1.3	+0.8	+0.1	-0.4	+0.1	-0.3	+0.3	+0.6	+5.6
	1931												
F 4.....	April 14	14.4	+1.3	+1.2	+0.3	-0.7	-0.7	-0.9	-0.8	-0.7	-1.3	-0.8	-3.2
F 5.....	April 24	16.2	+2.9	+2.4	+2.1	+1.0	0.0	+0.6	+2.0	+2.4	+0.7	+0.7	+14.8
F 6.....	May 7	12.2	+1.5	+1.2	+0.8	+1.3	-0.1	0.0	-0.5	-0.2	-0.1	+0.3	+4.4
Average.....		13.9	+1.7	+1.4	+1.1	+0.4	+0.1	0.0	+0.2	+0.3	-0.1	+0.2	+5.4

* For 15 minutes.

** Total change from base line computed from the metabolism for the entire experiment by means of the total oxygen absorption and the average respiratory quotient.

† Average of 3 experiments. See preceding article, page 635.

the males, M-3, gave a significant increase in heat production. Similarly in the following three periods, the increases with the females were slightly greater than with the males. After one hour, there is but little change in either direction for most of the periods with both groups of subjects. The total change in two and one-half hours varied widely in both groups of subjects. With the males, M-2 had a decrease of 3.4 calories in two and one-half hours as compared with the average base-line value for that period of time, whereas M-3 had an increase of 6.8 calories. The average of the 4 men was an increase of 2.2 calories for two and one-half hours. The

changes in the heat production of the females varied from -3.2 to $+14.8$, a wide range in heat production as the result of the ingestion of 20 grams of galactose. The results show that the heat production of the women varied much more in respect to the ingestion of galactose than did that of the men.

The changes in heat production as the result of the ingestion of 30 grams are given in table 8. With all of the males there was a uniform increase in the heat production in the first 6 periods, that is, one and one-half hours after the ingestion of the galactose. Even in subsequent periods there

TABLE 8

Changes from base line in the heat production of men and women after ingestion of 30 grams galactose

SUBJECT	DATE	BASE LINE*	PERIODS AFTER INGESTION OF GALACTOSE										Total change**
			1	2	3	4	5	6	7	8	9	10	
			0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.	
		cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.	cals.
Males:	1930												
M 2.....	July 17	15.9	+0.4	+1.2	+1.5	+1.3	+0.5	+0.6	-0.2	+0.1	+0.1	+0.5	+6.1
M 3.....	July 18	19.5	+2.3	+1.9	+2.2	+1.6	+1.2	+0.4	-0.3	0.0	+1.0	+0.8	+11.2
M 4.....	July 21	19.5	+1.2	+2.1	+2.5	+1.4	+0.4	+1.4	+0.5	+0.5	+1.4	+1.5	+12.7
Average.....		18.3	+1.3	+1.7	+2.1	+1.4	+0.7	+0.8	0.0	+0.2	+0.8	+0.9	+10.0
M 5†.....		16.1	+1.0	+2.2	+1.7	+1.1	+1.0	+0.6	+0.4	+0.2	+0.1	+0.3	+8.8
Females:	1930												
F 1.....	July 29	12.4	+1.0	+1.1	+1.3	+2.1	+1.2	+1.5	+0.6	+0.9	+0.3	+0.8	+11.1
F 3.....	July 30	14.0	+1.8	+1.7	+1.9	+2.0	+1.2	+0.6	+1.2	-0.6	-0.2		+9.8
	1931												
F 2.....	May 27	14.7	+1.9	+1.2	+0.7	+1.2	+0.5	+0.1	-0.4	-0.1	-0.4	-0.2	+4.5
F 4.....	April 28	13.5	+1.1	+1.4	+0.6	0.0	-0.5	-0.7	-0.8	-0.3	+0.2	0.0	+1.1
F 6.....	May 14	13.2	+0.6	+0.1	+0.9	+0.5	+0.3	0.0	+0.6	-0.3	-0.1	-0.4	+2.2
Average.....		13.6	+1.3	+1.1	+1.1	+1.2	+0.5	+0.3	+0.2	-0.1	-0.1	+0.1	+5.7

* For 15 minutes.

** Total change from base line computed from the metabolism for the entire experiment by means of the total oxygen absorption and the average respiratory quotient.

† Average of 2 experiments. See preceding article, page 635.

was a slight increase, varying from 0.1 to 1.5 calorie. M-5 showed an increase in heat production somewhat similar to the changes with the other 3 males. With the females there were increases in heat production through the first three 15-minute periods for all of the subjects. Subsequently there were varying results. Subjects F-1 and F-3 continued to show an increase in heat production through the seventh period (90 to 105 min.) after ingestion, whereas the other 3 subjects at this period of time had either returned to base line or else the values were below the base-line heat production. The maximum increase in heat production did not occur with

the group as a whole in the first period as it did with 20 grams. With F-1 and F-3 it was in the fourth period, with F-2 in the first period, and with F-4 in the second. F-6 differs from the others in that the increases in heat production were much lower during the first 3 periods. The total increase in heat production with the males varied from 6.1 to 12.7 calories, with an average of about 9.7 calories for the 4 males. Only 2 of the females showed increases in heat production approaching near these values, namely, F-1 and F-3. The other 3 subjects had low increases in heat production, even lower than occurred with 20 grams. Part of the low values can be ascribed to the negative changes in the last 4 or 5 periods of the experiments. The range is much wider than with the males, namely, from 1.1 to 11.1 with a general average of 5.7 calories, which is a little over one-half the average increase for the males.

The initial rise in energy output with the women may have been due to apprehension of the unknown effect of galactose, as the 20-gram experiments with the women were the first that they underwent. The second experiments were with 30 grams and they may not have reacted in the same way. To be sure, the same was true with the men, but our general impression was that the experiments were not so irksome and boresome to the men as to the women.

SUMMARY

The respiratory exchange was measured with an open-circuit apparatus and helmet for ten 15-minute periods with 5 men and 6 women after the ingestion of 20 grams of galactose in 200 cc. water at 37°C. and with 4 men and 5 women after the ingestion of 30 grams of galactose.

The average maximum rise in the respiratory quotient with 4 men was 0.11 in the third quarter-hour after ingestion, and 0.14 with the fifth subject (3 expts.) in the fourth quarter-hour with 20 grams. The female subjects had the same average rise as the 4 men, but a much greater variability individually. The average maximum rise after the ingestion of 30 grams was 0.16 with the men in the third and fourth quarter-hours and 0.17 with women in the fourth quarter-hour. Four out of 5 women had a maximum rise of 0.19 or over, thus greater than with any of the men.

The average increase in apparent combustion of carbohydrate after the ingestion of 20 grams of galactose was 4 grams with both sexes, but the variation with the females, 1.4 to 6.3 grams, was greater than with the males. The average increase with 30 grams was 9.9 grams with the males and 7.8 grams with the females.

The heat production after the ingestion of 20 grams of galactose increased 1.9 calories with 5 men and 5.4 calories with 6 women. After the ingestion of 30 grams, the heat production was raised 9.7 calories with 4 men and

5.7 calories with 5 women. The individual variations were much greater with the women than with the men.

The most marked differences in the two sexes was the tendency for the majority of the females to have higher maximum rises in respiratory quotient, greater increases in heat production at first after 20 grams, the greater increase in carbohydrate combustion and heat production with the males after 30 grams, and the generally wider deviations individually in all the factors with the women.

We wish to acknowledge our indebtedness to Dr. Allan Winter Rowe of the Evans Memorial Hospital, Boston, for providing us with the galactose and for arrangements for the subjects, and to the members of his staff who volunteered as subjects.

BIBLIOGRAPHY

- BENEDICT, F. G. 1930. *New England Journ. Med.*, cciii, 150.
BENEDICT, F. G. AND L. E. EMMES. 1915. *Journ. Biol. Chem.*, xx, 253.
BENEDICT, F. G. AND G. MACLEOD. 1929. *Journ. Nutr.*, i, 367.
BENEDICT, F. G. AND F. B. TALBOT. 1921. *Carnegie Inst. Wash. Pub.* 302.
BENEDICT, S. R. 1908. *Journ. Biol. Chem.*, v, 485.
1911. *Journ. Biol. Chem.*, ix, 57.
CARPENTER, T. M. AND R. C. LEE. 1932. *This Journal*, cii, 635.
DEUEL, H. J., JR. AND M. GULICK. 1932. *Journ. Biol. Chem.*, xcvi, 25.
GREISHEIMER, E. M. 1931. *Journ. Nutr.*, iv, 411.
HARDING, V. J. AND O. MOBERLEY. 1930. *Journ. Biol. Chem.*, lxxxix, 535.
MITCHELL, H. H., L. E. CARD AND W. T. HAINES. 1927. *Journ. Agric. Res.*, xxxiv, 945.
MITCHELL, H. H. AND W. T. HAINES. 1927. *Journ. Agric. Res.*, xxxiv, 927.
RIDDLE, O., G. CHRISTMAN AND F. G. BENEDICT. 1930. *This Journal*, xcv, 111.
ROE, J. H. AND A. S. SCHWARTZMANN. 1932. *Journ. Biol. Chem.*, xcvi, 721.
ROWE, A. W. 1924. *Arch. Int. Med.*, xxxiv, 388.

A COMPARISON OF THE EFFECTS ON THE HUMAN RESPIRATORY EXCHANGE OF HEXOSES INGESTED SEPARATELY AND TOGETHER¹

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Although the effects of simultaneous ingestion of two hexoses upon their absorption, on the height of blood sugar rise, and on the excretion of sugar in the urine have been the subjects of a number of investigations, practically nothing is known or can be predicted regarding the effect of ingestion of two sugars at the same time on the total metabolism, either qualitatively or quantitatively, as measured by the respiratory quotient and the total gaseous exchange, respectively. Deuel (1927) determined in man the respiratory quotient and the specific dynamic action following the ingestion of 37.5 grams of glucose plus 37.5 grams of fructose and compared them with the results obtained from the ingestion of 75 grams of sucrose. He found practically the same results in both cases, but as each hexose was not given in a separate experiment, he was unable to ascribe the rises in quotient and metabolism to either component. The studies of Carpenter and Fox (1930a, b) showed that small quantities of glucose and of fructose (10 grams or more) each produced characteristic changes in respiratory quotient and heat production, and the study of Carpenter and Lee (1932a) demonstrated that the ingestion of small quantities of galactose caused changes in respiratory quotient and total metabolism somewhat like those resulting from the ingestion of fructose. Under conditions of ordinary dietetic practices, these sugars are not given separately but in combinations. It is therefore of practical value as well as academic interest to determine whether each sugar maintains its characteristic reaction qualitatively when ingested together with the other sugars, and whether the effects on the heat production are the same. Are the effects on the respiratory quotient additive when the sugars are given together, that is, do they equal the sum of the effects when they are given separately? Is the resultant change in heat production the same as, greater than, or less than the

¹A preliminary communication was given at the meeting of the XIVth International Physiological Congress, Rome, Sept. 2, 1932. *Sunti delle comunicazioni scientifiche.* 1932. 48.

sum of the changes in heat production after the ingestion of the sugars separately?

To secure information regarding these problems, several series of experiments were made with a human subject, in which the effects of the sugars, glucose, fructose, and galactose when given separately were compared with the effects when given together. Twenty-gram amounts were used, and first a series of experiments with each hexose alone was made, and then combinations of 20 grams each of glucose and fructose, glucose and galactose, fructose and galactose were ingested. Two experiments with 40 grams of lactose were also made to compare the effect of a disaccharide with the effect of its equivalent amount of hydrolysis products, glucose and galactose. The sugars were dissolved in 200 cc. of water at 37°C., and the same volume of water was used whether 20 or 40 grams of sugar were ingested. The procedure of an experiment and the apparatus used were the same as in the two preceding investigations. (Carpenter and Lee, 1932a, b.)

RESULTS OF EXPERIMENTS. The respiratory quotients determined in these experiments are recorded in table 1. The date and the amount and kind of sugar ingested are given in the first column. In the second column are the base-line respiratory quotients, and in the next ten columns the quotients measured following the sugar ingestion. The last column gives the average change from the base line for the whole experiment of two and one-half hours. The average change from the base line in any one group of experiments is likewise shown for each of the ten periods after sugar ingestion.

The amounts of carbohydrate metabolized or burned in the base-line periods and the changes from the base-line amounts after the ingestion of 20 grams each of glucose, fructose, and galactose and combinations of these sugars are recorded in table 2. These values were calculated from the respiratory quotient and oxygen absorption without correcting for protein. The last column gives the total change from the base-line value for the period of two and one-half hours, calculated not by adding the amounts of the individual changes in periods 1 to 10, but from the total carbon-dioxide output and the total oxygen consumption in two and one-half hours. This method gives a more exact figure than the addition of the values in the ten columns, because it does away with the slight variations due to the reduction of the number of decimal places, and also because in the experiments with combinations of fructose and galactose, there were many periods in which the respiratory quotient was higher than unity. When the calculation of the metabolism of carbohydrates was made by periods, it was assumed that the respiratory quotient was unity or below.

The average heat production in the base-line periods and the changes from the base line in 10 successive 15-minute periods are given in table 3.

In these three tables, for purposes of easy reference, Roman numerals

TABLE 1

Respiratory quotients before and after the ingestion of hexoses and combinations of hexoses (Mr. J. C.)

DATE (1931)		BASE LINE	PERIODS AFTER INGESTION OF SUGARS										Aver- age change
			1	2	3	4	5	6	7	8	9	10	
			0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.	
20 grams glucose (I):													
May 11.....	0.89	0.87	0.91	0.93	0.93	0.89	0.89	0.89	0.88	0.87	0.87	+0.005	
October 14.....	0.83	0.83	0.84	0.84	0.89	0.89	0.88	0.88	0.86	0.87	0.86	+0.030	
October 16.....	0.82	0.80	0.81	0.84	0.86	0.86	0.84	0.84	0.82	0.82	0.82	+0.015	
Average change from base line.....			-0.01	+0.01	+0.02	+0.05	+0.03	+0.03	+0.03	+0.01	+0.01	0.00	+0.015
20 grams fructose (II):													
March 28.....	0.80	0.80	0.89	0.91	0.92	0.90	0.87	0.86	0.85	0.85	0.83	+0.065	
March 30.....	0.81	0.82	0.90	0.94	0.88	0.88	0.83	0.81	0.78	0.82	0.80	+0.040	
October 12.....	0.84	0.84	0.97	0.95	0.94	0.87	0.86	0.83	0.83	0.82	0.82	+0.035	
Average change from base line.....			0.00	+0.11	+0.12	+0.10	+0.07	+0.04	+0.01	+0.01	+0.01	0.00	+0.045
20 grams galactose (III):													
March 18.....	0.84	0.83	0.91	0.98	0.97	0.86	0.86	0.80	0.79	0.80	0.80	+0.020	
March 23.....	0.82	0.82	0.85	0.94	1.01	0.90	0.89	0.85	0.86	0.83	0.84	+0.055	
October 9.....	0.86	0.84	0.88	0.96	0.96	0.89	0.88	0.87	0.84	0.85	0.83	+0.020	
Average change from base line.....			-0.01	+0.04	+0.12	+0.14	+0.04	+0.04	0.00	-0.01	-0.01	-0.02	+0.030
20 grams glucose and 20 grams fructose to- gether:													
April 3.....	0.87	0.90	1.05	1.00	0.95	0.91	0.97	0.96	0.93	0.92	0.88	+0.075	
October 19.....	0.87	0.87	1.01	0.99	0.89	0.92	0.92	0.91	0.86	0.89	0.87	+0.045	
Average change from base line.....			+0.01	+0.16	+0.13	+0.05	+0.05	+0.08	+0.07	+0.02	+0.03	+0.01	+0.060
(I) + (II).....			-0.01	+0.12	+0.14	+0.15	+0.10	+0.07	+0.04	+0.02	+0.02	0.00	+0.065
20 grams galactose and 20 grams fructose to- gether:													
April 1.....	0.82	0.80	0.99	1.05	0.97	0.90	0.88	0.90	0.82	0.81	0.80	+0.075	
April 6.....	0.88	0.88	1.05	1.02	0.95	0.91	0.88	0.87	0.82	0.81	0.84	+0.025	
April 17.....	0.82	0.83	0.97	1.05	0.97	0.91	0.91	0.89	0.87	0.86	0.85	+0.090	
May 13.....	0.79	0.81	0.97	1.02	0.94	0.90	0.89	0.91	0.82	0.82	0.81	+0.100	
Average change from base line.....			0.00	+0.17	+0.21	+0.13	+0.08	+0.07	+0.07	+0.01	0.00	0.00	+0.075
(II) + (III).....			-0.01	+0.15	+0.24	+0.24	+0.11	+0.08	+0.01	0.00	0.00	-0.02	+0.080
20 grams galactose and 20 grams glucose to- gether:													
April 15.....	0.81	0.81	0.84	0.92	0.96	0.93	0.90	0.86	0.86	0.83	0.83	+0.060	
April 21.....	0.86	0.85	0.92	1.02	1.00	0.96	0.91	0.87	0.86	0.85	0.86	+0.050	
April 27.....	0.83	0.82	0.89	0.96	1.00	0.94	0.88	0.86	0.87	0.85	0.83	+0.060	
Average change from base line.....			-0.01	+0.05	+0.13	+0.15	+0.11	+0.06	+0.03	+0.03	+0.01	+0.01	+0.055
(I) + (III).....			-0.02	+0.05	+0.14	+0.19	+0.07	+0.07	+0.03	0.00	0.00	-0.02	+0.050
40 grams lactose:													
April 30.....	0.87	0.84	0.90	0.97	0.98	0.95	0.93	0.90	0.91	0.89	0.88	+0.045	
May 15.....	0.84	0.84	0.89	0.97	0.98	0.95	0.91	0.90	0.88	0.88	0.87	+0.065	
Average change from base line.....			-0.02	+0.04	+0.12	+0.12	+0.09	+0.06	+0.04	+0.04	+0.03	+0.02	+0.055

TABLE 2

The metabolism of carbohydrates as affected by the ingestion of hexoses and combinations of hexoses (Mr. J. C.)

DATE (1931)	BASE LINE*	CHANGES FROM BASE LINE IN PERIODS AFTER INGESTION OF SUGARS											Total change **
		1	2	3	4	5	6	7	8	9	10		
		0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.		
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	
20 grams glucose (I):													
May 11.....	2.5	-0.2	+0.5	+0.8	+0.6	0.0	0.0	-0.1	-0.3	-0.3	-0.3	+0.2	
October 14.....	1.6	+0.1	+0.2	+0.2	+0.8	+0.7	+0.6	+0.6	+0.3	+0.4	+0.4	+3.9	
October 16.....	1.5	-0.2	-0.1	+0.3	+0.6	+0.5	+0.2	+0.2	0.0	-0.1	-0.1	+1.3	
Average.....	1.9	-0.1	+0.2	+0.4	+0.7	+0.4	+0.3	+0.2	0.0	0.0	0.0	+1.8	
20 grams fructose (II):													
March 28.....	1.4	-0.1	+1.3	+1.5	+1.6	+1.3	+0.8	+0.6	+0.5	+0.5	+0.3	+8.5	
March 30.....	1.4	+0.4	+1.4	+2.0	+1.3	+1.2	+0.5	+0.2	-0.3	—	0.0	+7.2†	
October 12.....	1.7	+0.1	+1.9	+1.5	+1.3	+0.4	+0.2	-0.2	-0.2	-0.3	-0.3	+3.7	
Average.....	1.5	+0.1	+1.5	+1.7	+1.4	+1.0	+0.5	+0.2	0.0	+0.1	0.0	+6.5	
20 grams galactose (III):													
March 18.....	1.6	-0.2	+1.2	+1.9	+1.7	+0.2	+0.2	-0.5	-0.8	-0.5	-0.6	+2.7	
March 23.....	1.3	0.0	+0.4	+1.5	+1.9	+1.0	+0.8	+0.3	+0.4	+0.1	+0.1	+7.6	
October 9.....	1.7	-0.3	+0.4	+1.5	+1.3	+0.4	+0.2	0.0	-0.3	-0.2	-0.5	+2.7	
Average.....	1.5	-0.2	+0.7	+1.6	+1.6	+0.5	+0.4	-0.1	-0.2	-0.2	-0.3	+4.3	
20 grams glucose and 20 grams fructose to- gether:													
April 3.....	2.2	+0.6	+2.0	+2.1	+1.2	+0.5	+1.4	+1.0	+0.7	+0.6	+0.3	+11.5	
October 19.....	2.1	+0.1	+2.2	+2.1	+0.3	+0.6	+0.6	+0.5	-0.2	+0.1	-0.2	+6.8	
Average.....	2.2	+0.3	+2.1	+2.1	+0.7	+0.5	+1.0	+0.7	+0.2	+0.3	+0.1	+9.2	
(I) + (II).....		0.0	+1.7	+2.1	+2.1	+1.4	+0.8	+0.4	0.0	+0.1	0.0	+8.3	
20 grams galactose and 20 grams fructose to- gether:													
April 1.....	1.6	-0.2	+2.9	+3.1	+2.5	+1.3	+1.0	+1.2	0.0	-0.1	-0.3	+12.7	
April 6.....	2.3	+0.2	+2.1	+2.3	+1.3	+0.6	0.0	-0.2	-0.8	-1.0	-0.7	+5.0	
April 17.....	1.5	+0.3	+2.3	+2.8	+2.3	+1.3	+1.4	+1.0	+0.7	+0.5	+0.4	+13.2	
May 13.....	1.2	+0.3	+2.8	+3.2	+2.2	+1.5	+1.4	+1.7	+0.4	+0.4	+0.2	+14.1	
Average.....	1.7	+0.1	+2.5	+2.8	+2.1	+1.2	+0.9	+0.9	+0.1	-0.1	-0.1	+11.3	
(II) + (III).....		-0.1	+2.2	+3.3	+3.0	+1.5	+0.9	+0.1	-0.2	-0.1	-0.3	+10.8	
20 grams galactose and 20 grams glucose to- gether:													
April 15.....	1.4	+0.1	+0.6	+1.8	+2.4	+1.8	+1.3	+0.6	+0.6	+0.2	+0.2	+10.2	
April 21.....	2.0	0.0	+1.1	+2.2	+2.3	+1.7	+0.9	+0.2	0.0	-0.2	-0.1	+8.2	
April 27.....	1.7	0.0	+1.0	+2.3	+2.7	+1.7	+0.8	+0.4	+0.5	+0.2	-0.1	+9.2	
Average.....	1.7	0.0	+0.9	+2.1	+2.5	+1.8	+1.0	+0.4	+0.4	+0.1	0.0	+9.2	
(I) + (III).....		-0.3	+0.9	+2.0	+2.3	+0.9	+0.7	+0.1	-0.2	-0.2	-0.3	+6.1	
40 grams lactose:													
April 30.....	2.2	-0.4	+0.6	+1.9	+1.8	+1.3	+1.0	+0.4	+0.5	+0.1	+0.1	+7.8	
May 15.....	1.8	0.0	+0.8	+2.1	+2.3	+1.8	+1.2	+1.0	+0.6	+0.5	+0.4	+10.8	
Average.....	2.0	-0.2	+0.7	+2.0	+2.0	+1.6	+1.1	+0.7	+0.6	+0.3	+0.2	+9.3	

* For 15 minutes.

** Computed from the total oxygen and the average respiratory quotient for the entire 2½ hours after the sugars.

† For periods 1-8 and 10.

I to III have been assigned to the three series of experiments with the single sugars, and the average period changes from the base line in the series of experiments with combinations of sugars have been compared with the sum of the average period changes in the experiments with single sugars, designated as "(I) + (II)," and so forth. There are also slight discrepancies between the averages and totals given in the tables because the values were taken from the original protocols that were calculated to more significant figures than are given in these tables.

The experiments continued for $2\frac{1}{2}$ hours after the ingestion of the sugar and in the tables the values are given for all of the periods whether or not there was a positive effect for the $2\frac{1}{2}$ hours. The length of time that an increase above base line was found, varies in each group of experiments, and the number of periods in which minus values are found also varies with each group of experiments. The results may be handled in two ways, that is, the values which are positive and which indicate a rise above the base line may be utilized, or the values for the whole period of time may be employed. In the latter case, negative values come into the discussion as well as positive values.

The duration of $2\frac{1}{2}$ hours was employed because it had been found in previous experiments that for the amounts used most of the effect, if not all, was over in $2\frac{1}{2}$ hours, but it has also been found in previous experiments that when varying amounts of sugars are given the negative values after the rise above the base line is completed are not of the same magnitude with each succeeding increasing amount of sugar. Therefore, in order to make a strict comparison one should take into account not only the rises above the base line, but also the negative values below the base line for the total $2\frac{1}{2}$ hours.

In the following discussion, groups of experiments are compared with one another for the total $2\frac{1}{2}$ hours as well as for the succeeding periods. This is done in order to have all the experiments on a comparable basis as far as the duration is concerned. It, however, brings in the apparently anomalous situation that the sum of the rises in the first few periods of the experiment may be greater than the total net change for the $2\frac{1}{2}$ hours, which includes the negative changes in the later portions of the experiment.

Twenty grams of glucose. The first group of experiments was with 20 grams of glucose. The maximum rise in the respiratory quotient in any single period was 0.06 on October 14 in periods 4 and 5. From period 5 to period 10 there was a gradual fall to the base line. The findings in this group are comparable to those in previous studies with about the same amount of glucose. The maximum rise in carbohydrate metabolized was during the fourth period. Thereafter there was a drop to nearly the base-line value in period 7. The average total change calculated from the whole of the experiments is +1.8 grams. The maximum rise in heat pro-

TABLE 3

The heat production as affected by the ingestion of hexoses and combinations of hexoses (Mr. J. C.)

DATE (1931)	BASE LINE*	CHANGES FROM BASE LINE IN PERIODS AFTER INGESTION OF SUGARS										TOTAL CHANGE **
		1	2	3	4	5	6	7	8	9	10	
		0 to 15 min.	15 to 30 min.	30 to 45 min.	45 to 60 min.	60 to 75 min.	75 to 90 min.	90 to 105 min.	105 to 120 min.	120 to 135 min.	135 to 150 min.	
	cal.	cal.	cal.	cal.	cal.	cal.	cal.	cal.	cal.	cal.	cal.	cal.
20 grams glucose (I):												
May 11.....	16.9	+0.4	+1.4	+1.4	+0.6	+0.2	+0.2	-0.7	-0.9	-0.3	-0.4	+1.6
October 14.....	15.6	+0.9	+0.9	+0.9	+0.2	-0.2	-0.3	-0.1	-0.4	-0.5	+0.1	+1.3
October 16.....	16.2	+0.3	+0.9	+0.7	+0.3	-0.3	-0.4	-0.5	-0.2	-0.6	-0.6	-0.3
Average.....	16.2	+0.5	+1.1	+1.0	+0.4	-0.1	-0.2	-0.4	-0.5	-0.5	-0.3	+0.9
20 grams fructose (II):												
March 28.....	18.1	-0.8	-0.3	-0.3	-0.7	-1.1	-1.7	-2.0	-1.7	-2.3	-1.7	-12.6
March 30.....	17.1	+2.6	+1.1	+0.9	+2.5	+1.9	+2.5	+2.5	+2.7	-	+2.2	+19.0†
October 12.....	16.1	+0.5	+1.2	+0.2	+0.1	-0.2	-0.7	-0.7	-0.6	-0.6	-0.9	-1.8
Average.....	17.1	+0.8	+0.7	+0.2	+0.6	+0.2	0.0	-0.1	+0.2	-1.5	-0.1	+1.5
20 grams galactose (III):												
March 18.....	16.6	+0.5	+1.8	+1.0	+0.1	+0.1	-0.2	+0.3	0.0	+0.2	-0.1	+3.8
March 23.....	15.9	+0.3	+0.8	+0.7	+0.4	-0.5	-0.2	-0.7	-0.4	0.0	-0.2	+0.6
October 9.....	15.5	+0.1	+1.5	+1.0	-0.1	-0.8	-0.9	-1.0	-0.6	-0.7	-1.1	-2.5
Average.....	16.0	+0.3	+1.4	+0.9	+0.1	-0.4	-0.4	-0.5	-0.3	-0.2	-0.5	+0.6
20 grams glucose and 20 grams fructose to- gether:												
April 3.....	17.0	+0.9	+1.1	+1.4	+0.4	-0.5	+0.2	-1.2	-0.8	-0.8	+1.4	+2.6
October 19.....	15.9	+0.7	+2.2	+2.3	0.0	-0.3	-0.1	-0.4	-0.5	-0.9	-1.1	+2.2
Average.....	16.5	+0.8	+1.7	+1.8	+0.1	-0.4	0.0	-0.8	-0.7	-0.8	+0.1	+2.4
(I) + (II).....		+1.3	+1.8	+1.2	+1.0	+0.1	-0.2	-0.5	-0.3	-2.0	-0.4	+2.4
20 grams galactose and 20 grams fructose to- gether:												
April 1.....	16.9	+1.3	+2.4	+2.7	+2.2	+1.0	+1.3	+0.5	+0.1	0.0	-0.3	+11.6
April 6.....	16.8	+1.2	+2.1	+2.8	+1.9	+1.1	0.0	-0.2	-0.7	-1.1	-1.3	+6.0
April 17.....	16.6	+1.1	+1.4	+1.6	+1.2	+0.8	+0.9	+0.2	+0.1	-0.3	+0.2	+7.6
May 13.....	17.2	-0.4	+1.5	+1.2	+0.7	-0.3	-0.1	+0.3	-0.2	-0.5	-0.7	+1.8
Average.....	10.9	+0.8	+1.9	+2.1	+1.5	+0.6	+0.5	+0.2	-0.2	-0.4	-0.5	+6.8
(II) + (III).....		+1.1	+2.1	+1.1	+0.7	-0.2	-0.4	-0.6	-0.1	-1.7	-0.6	+2.1
20 grams galactose and 20 grams glucose to- gether:												
April 15.....	16.4	+0.8	+1.7	+1.8	+1.9	+1.4	+0.9	-0.2	-0.6	-0.6	-0.7	+6.5
April 21.....	16.5	+1.5	+1.7	+1.6	+2.0	+2.1	+1.3	+0.3	+0.3	-0.4	-0.7	+9.6
April 27.....	16.6	+1.3	+1.7	+2.8	+2.0	+1.4	+1.0	-0.2	-0.6	-0.4	-1.0	+7.9
Average.....	16.5	+1.2	+1.7	+2.1	+2.0	+1.6	+1.1	0.0	-0.3	-0.5	-0.8	+8.0
(I) + (III).....		+0.8	+2.5	+1.9	+0.5	-0.5	-0.6	-0.9	-0.8	-0.7	-0.8	+1.5
40 grams lactose:												
April 30.....	16.6	+0.5	+1.3	+2.4	+1.4	+1.3	+0.9	+0.1	+0.1	-1.0	-0.4	+6.7
May 15.....	16.9	+0.1	+0.6	+1.4	+1.6	+1.6	+1.3	+1.1	+0.3	-0.1	-0.2	+7.7
Average.....	16.8	+0.3	+0.9	+1.9	+1.5	+1.4	+1.1	+0.6	+0.2	-0.5	-0.3	+7.2

* For 15 minutes.

** Computed from total oxygen and average respiratory quotient for the entire 2½ hours after the ingestion of the sugars.

† Total for periods 1-8 and 10.

duction was 1.4 calories (8 per cent of the base-line value of 16.9 calories) in periods 2 and 3 on May 11, and the average maximum rise was 1.1 calories (9 per cent) in period 2. This is a half-hour earlier than the maximum rise in carbohydrate combustion (see table 2, period 4). The ingested sugar would supply the energy for a little more than 1 hour. The sum of the increases in the first 4 periods is 3 calories, i.e., 4 per cent of the heat of combustion of the ingested glucose. The average net change in heat production in two and one-half hours was +0.9 calorie.

Twenty grams of fructose. The maximum rise of 0.13 in the respiratory quotient was in period 2 on October 12 and period 3 on March 30. This corresponds to the time when the maximum increase in respiratory quotient occurred in most of the earlier studies of the effect of the ingestion of fructose. The greatest effect in any single experiment was on March 28, where the return to the base line is not so marked as in the other two experiments. The group as a whole shows a fairly rapid return to base-line level. The average rise in quotient for two and one-half hours was 0.045, three times that after glucose. The maximum average rise in the combustion of carbohydrates was in period 3, and from there on until the end of the experiment there was a gradual fall. The rise is larger than with the same weight of glucose and the average total change in carbohydrate metabolized for the whole experiment is +6.5 grams, over three times that for glucose. The 3 experiments with 20 grams of fructose gave different results in the heat production in the individual experiments. On March 28, the base-line value was the highest of any in the series, and all of the periods after ingestion gave lower heat production than the base line. On the contrary, on March 30, all of the periods after ingestion gave increases in heat production in spite of the fact that the base-line value (17.1 cal.) was the second highest in the series. This experiment has been subjected to a theoretical checking by measuring the total ventilation and calculating the composition of expired air from the base-line values with special reference to the percentage of carbon dioxide, which has been found in earlier experiments of this sort (Carpenter and Fox, 1931) to be nearly constant. The average percentage of the base-line periods was then applied to the measured ventilation, and by means of the respiratory quotient the theoretical oxygen absorption was estimated. The calculated values were close enough to those determined to be able to conclude that the observed values were correct. There is no reason to reject the experiment. One has to assume that the dose produced an unusual reaction or that some unknown condition caused a rise in the metabolism. The experiment on October 12 appears to be nearer to the probable effect of ingestion of fructose than the other two. The summation of the average changes in periods 1 to 5 inclusive is 2.5 calories, 3 per cent of the energy content of the ingested fructose. In a previous series (Carpenter and Fox, 1930b), the

increase in heat production after 21 grams of fructose was 0.8 calorie in one hour, thus lower than the group here studied. Reference to table 2 shows that in spite of the lack of uniformity in the results of the calculated heat production, the changes in carbohydrate combustion were, for the most part, positive and much more uniform than the changes in heat production. The respiratory quotient is the determining element in carbohydrate combustion and not the heat production. The course of the combustion of carbohydrate appears to be independent of the heat production.

Twenty grams of galactose. The three experiments with 20 grams of galactose have been given in a preceding publication (Carpenter and Lee, 1932a) but are here repeated for comparison with the other sugars. The maximum rise in the respiratory quotient was 0.19 in period 4 on March 23. The average maximum rise was 0.14 and was one quarter-hour later than with fructose and the same period of time as with glucose. From this period on until the seventh period there was a sharp drop in the respiratory quotient so that all of the periods after the seventh are below the base line with respect to the respiratory quotient. This change to below the base-line level is different from the changes noted with the other two sugars and would point to a slight compensation for over-ventilation during some of the preceding periods. We have no indication as to the effect of galactose upon the alkali reserve in this group of experiments, but Campbell and Maltby (1928) have found that there was no change in the alkali reserve or in the lactic acid of the blood after the ingestion of galactose. Galactose and fructose resemble one another in the effects on the respiratory quotient more than glucose resembles either one of the other two sugars. The maximum rise in combustion of carbohydrate occurred at the same time in this group as with fructose and was nearly of the same order, but both preceding and following these periods the values are somewhat lower than those of fructose. In the last four periods the average change is negative. The summation of the average increases in heat production in periods 1 to 4 is 2.7 calories, 4 per cent of the energy value of the galactose.

The three sugars, glucose, fructose, and galactose in 20-gram doses, produced about the same amount of increase in heat production and the period of maximum increase was in the second quarter-hour after ingestion. The amounts ingested are probably too small to show a differentiation in effects among the three sugars.

Twenty grams of glucose + 20 grams of fructose. The effects on the respiratory quotient of the ingestion of this sugar combination can best be ascertained by comparing the sum of the increases in the respiratory quotients when the two sugars are given separately with the increases when the two sugars are given together. In periods 1, 2, and 3 there is very little difference between these two sets of figures. The sum of the two produces

a more marked effect upon the respiratory quotient, namely, 0.15, 0.10, and 0.07 in periods 4, 5, and 6, as against 0.05, 0.05, and 0.08 when the sugars are given together. The average change in the respiratory quotient for two and one-half hours was $+0.060$ in the case of the sugars given together as compared with $+0.065$, the summation of the effect of the two sugars ingested separately. The maximum rise in combustion of carbohydrates occurred in periods 2 and 3. When the effects of these two sugars given separately are added, periods 2 and 3 give an increase of 1.7 and 2.1, respectively, nearly the same as when given together. The total change when 20 grams of glucose and 20 grams of fructose are given separately is 8.3 grams compared with 9.2 found when they are given together. The maximum increase in heat production was 2.3 calories (15 per cent of the base-line value, 15.9 calories) in period 3 on October 19. In both experiments there were marked increases in the first three periods. The average net change in two and one-half hours was 2.4 calories, a value equal to the sum of the net changes of the same sugars given separately.

Twenty grams of galactose + 20 grams of fructose. There was a marked rise in the respiratory quotient in periods 2 and 3, which exceeded or nearly equalled the sum of the effects of the two sugars given separately, but in period 4 the sum of the effects of the two sugars was twice the effect when given together. Thereafter there was not much difference between the two sets of figures as they both return to a base-line level in period 8. The average change in both conditions was nearly the same. The maximum rise in combustion of carbohydrates occurred in period 3, and there was a return, on the average, to base-line level by period 8. The effects of the ingestion of each of these sugars separately, added together by periods, give values not far from those when the two sugars are given together. The maximum difference is in period 4, where the sum is equal to 3.0 grams as compared with 2.1 when given together and $+0.1$ in period 7 as compared to $+0.9$. The sum of the total changes in combustion of carbohydrates when the sugars are given separately is equal to 10.8 grams as compared with 11.3 when they are given together. The maximum increase in heat production was 2.8 calories (17 per cent) in period 3 of April 6, and the average change for the same period was $+2.1$ calories. The average net change in two and one-half hours was $+6.8$ calories which is over 3 times the sum (2.1) of the effects of the two sugars given separately.

Twenty grams of galactose + 20 grams of glucose. The next group of experiments was with 20 grams of galactose and 20 grams of glucose, which theoretically are the products of hydrolysis of a nearly equal amount of lactose. A summation of the results obtained when the two sugars are given separately compared with the results when the two sugars are given together shows that there was little difference in the effects on the respiratory quotient. The maximum effect of $+0.15$ when two sugars are given

together is not so large as the sum (0.19) of the effects of the two sugars separately, but the subsequent period shows a larger rise under the first condition than under the latter and there is a greater after-effect, so to speak, when the two sugars are given together than when they are given separately, and their effects added. The average change in respiratory quotient for two and one-half hours was $+0.055$ when the sugars were given together as compared with $+0.050$, the sum when given separately. The maximum change in combustion of carbohydrates was in period 4 with a rapid return to a base-line level in period 9 and the experiments, as a whole, are uniform in all three series. The sum of the effects of 20 grams of galactose and of glucose is not so large when these sugars are given separately as when given together. For the first four periods the changes are practically the same but from there on they are somewhat less when the sugars are given separately than when given together. The total change for the two and one-half hours is 6.1 as compared with 9.2 grams. This comparison shows the greatest difference in combustion of carbohydrates of any of the comparisons which have been made. The maximum increase in heat production was 2.8 calories (17 per cent) in period 3 on April 27. The results in this group as a whole were more uniform than in any of the other groups. The average net change in two and one-half hours was 8.0 calories, over 5 times the sum (1.5 calories) of the effects of these two sugars given separately.

Forty grams of lactose. The last group of experiments was with 40 grams of lactose, which is practically equivalent chemically to the preceding group of experiments. The effect of 40 grams of lactose on the respiratory quotient was slightly less from periods 2 to 5 inclusive, than those of a combination of 20 grams each of glucose and galactose, although the difference is not large. From there on the effect of the lactose is slightly larger than the effect of an equivalent amount of the products of hydrolysis, and the return to the base line is not so rapid. In fact, the individual experiments in each group show greater differences in the changes than the differences between the two groups of averages and consequently we may conclude that the effect upon the respiratory quotient is nearly the same when 40 grams of lactose are given as when 20 grams of galactose and 20 grams of glucose are given. The increases in carbohydrate burned after the ingestion of 40 grams of lactose were practically the same as those when its equivalent in a hydrolyzed condition, namely, 20 grams of glucose and 20 grams of galactose, were given. There is a slightly greater effect in the last portion of the experiment when 40 grams of lactose were given than when its equivalent in hydrolyzed constituents were given. It would appear as though there was a slight delay in the effect on the metabolism when lactose is given, as shown by the smaller change in period 4 and the greater changes in periods 6 to 10, although the differences are not marked.

The total change in the two and one-half hours was 9.3 grams, which is practically the same as with the equivalent sugars in the hydrolyzed condition. The maximum increase in heat production in the experiments with 40 grams of lactose was 2.4 calories in period 3 on April 30. The changes in heat production by periods were not so large with 40 grams of lactose as with the equivalent 20 grams each of galactose and glucose, but the effect was more prolonged as the increase lasted one-half hour longer. The average net change in two and one-half hours was +7.2 calories, nearly as large as with the equivalent amount of glucose and galactose.

The results of the studies of the respiratory quotient show in general that when simple sugars are given together, they are additive in the main in the effects on the respiratory quotient and that therefore each sugar maintains its separate effect on the processes as represented by the respiratory quotient whether given singly or whether given in combinations.

In practically all of the groups the maximum change in carbohydrate occurs with the lower base-line level. For example: In the glucose experiments the increases on October 14 and 16 are somewhat larger than the increases on May 11. Similarly, with 20 grams of fructose the increases on March 28 and 30 are larger than the increases on October 12. On March 23, with 20 grams of galactose there is a much greater increase in carbohydrate catabolized than in the other two experiments. In the group with 20 grams of glucose and 20 grams of fructose, the base-line levels are practically identical, but the increases are different in the two experiments. In the four experiments with 20 grams of galactose and 20 grams of fructose, the maximum change occurs with the lowest base-line value, namely, on May 13, and the smallest change occurs with the highest base-line value, namely, on April 6. The same finding holds true with regard to the other two groups, that is, the lower the base-line combustion of carbohydrate is, the greater is the effect on the combustion of carbohydrate, which results from the ingestion of sugars. The effects hold true regardless of whether it is glucose, fructose, or galactose or whether the combinations of these sugars are used.

This finding is different than would be expected if the respiratory quotients in the base-line condition represent the supply of carbohydrate on hand in the body for combustion. One would expect that the lower the respiratory quotient the smaller would be the rise in the respiratory quotients, and consequently in the combustion of carbohydrates after the ingestion of the sugars. The range in respiratory quotients of the base line is from 0.79 to 0.88. The lower value does not indicate a marked depletion of carbohydrate reserves, but the higher value indicates a larger proportion of the combustion of carbohydrates than one would normally expect in the post-absorptive condition. It is possible that when the respiratory quotients are as high as 0.88 it is an indication of a condition which is

not stable, but one which would subsequently change with the respiratory quotient falling gradually. In that case the effect of the ingestion of sugar would not be so great as when the respiratory quotient is at the lower or more normal level of 0.79 to 0.82. Part of the effect of the ingestion of the sugars on the respiratory quotient and combustion of carbohydrate would thus be neutralized by the subsequent fall in respiratory quotient and combustion of carbohydrates when the initial or base-line quotient was high. A lack of rise in the respiratory quotient and in the combustion of carbohydrates would be more likely to take place if the base-line respiratory quotients were more nearly the value of almost exclusive fat combustion. This would not necessarily apply to all three hexoses as with fructose the metabolism may be one of conversion of sugar to fat instead of supplying the glycogen reserves.

Although the increases in respiratory quotients and the increases in combustion of carbohydrate are additive when two hexoses are ingested together, the heat production after combinations with galactose are ingested is greater than the sum of the changes in heat production when the sugars are given separately. This effect is greater than can be accounted for by 20 grams of galactose alone. It apparently is not due to the fact that the amount of sugar ingested is 40 grams instead of 20, as the 40-gram combination of fructose and glucose produces only the sum of the effects of these given separately. It can not be due to the more rapid absorption of galactose in the presence of other sugars, as previous investigations have shown just the contrary. Cori (1925) found with rats that the rate of absorption of galactose was less when galactose was combined with glucose than when it was ingested alone. Corley (1928) observed that the rise in unfermentable reducing substances in the blood and urine of rabbits after the ingestion of galactose alone was delayed when the galactose was combined with glucose, and that an analysis of the intestinal contents showed a delayed absorption of the galactose when glucose was given at the same time. Bodansky (1923) found that the hyperglycemia and the glycuressis that occurred after the ingestion of galactose in dogs were much diminished when an equal quantity of glucose was mixed with the galactose. The presence of the unabsorbed sugar (galactose) may be the cause of the greater heat production, although there is no evidence that unabsorbed nutrients stimulate heat production. It might be that more of the galactose is changed to lactic acid, which would act as a stimulant to metabolism. Wierzuchowski and Laniewski (1931) found that intravenous injection of galactose was followed by a greater rise in lactic acid of the blood than the injection of glucose. It may be that in the presence of other glycogen formers, less of the galactose is converted to glycogen and more of it is burned and that this transformation requires energy. These suggestions are wholly speculative and it is apparent that other experiments are needed

to find the cause of the greater heat production when galactose is combined with other sugars than when given alone.

SUMMARY

With the helmet open-circuit apparatus a study was made of the respiratory exchange of an adult man before and after the ingestion of 20 grams each of glucose, fructose, and galactose and after various combinations of two sugars in 20-gram amounts, each, during one hour previous to ingestion and for two and one-half hours after ingestion, in 15-minute periods.

Glucose and fructose together, galactose and fructose, and galactose and glucose given together produced nearly the same rise in respiratory quotient as the summation of the rises with the sugars given separately. Forty grams of lactose caused slightly smaller rises than the equivalent 20 grams of glucose and of galactose given together, but the return to the pre-ingestion values in the respiratory quotient was not so rapid with lactose as with glucose and galactose.

When glucose and fructose were given together, the increase in carbohydrate combustion averaged 9.2 grams as compared with 8.3, the sum of the increases when given separately. Galactose and fructose given together produced 11.3 grams increase in carbohydrate combustion as compared with 10.8 when given separately. Galactose and glucose given together brought about an average increase of 9.2 grams as compared with 6.1 when given separately. Forty grams of lactose produced an average increase of 9.3 grams of carbohydrate burned as compared with 9.2 when the two sugars equal to the hydrolysis products were given together.

The results were more uniform in all of the experiments with respect to the increases in respiratory quotient and the increases in carbohydrate combustion than they were with respect to the increases in heat production.

When 20 grams each of glucose and fructose were given together, the increase in heat production was 2.4 calories, equal to the summation of the effects of these sugars given separately. However, when 20 grams each of fructose and galactose were given together, the increase in heat production was 6.8 calories as compared with 2.1 when summated. Similarly, the increase in heat production of galactose and glucose given together was 8.0 as compared with 1.5 calories, the sum of the effects when given separately. Forty grams of lactose also produced an increase of 7.2 calories, thus equal to the increase in heat production of the products of hydrolysis of lactose when given separately.

The giving of 20 grams each of glucose, fructose, and galactose in combinations of two sugars thus produces practically the same increases in the respiratory quotient and in the combustion of carbohydrates as would be found by the addition of their effects when given separately. When galactose was combined with another sugar there was a greater increase in heat

production than would be expected from the summation of the effects of galactose and any other sugar when given separately.

It would appear that the qualitative reactions due to the ingestion of hexoses were the same whether given separately or together, but that some other factor than the changes in the transformations of the carbohydrates played a rôle in bringing about the increases in heat production when galactose was ingested with some other sugar. It is suggested that the cause may be the presence of more unabsorbed galactose in the alimentary tract when other sugars are ingested, or the greater formation of lactic acid in the intermediary metabolism of galactose, or the smaller formation of glycogen when other glycogen formers are available.

BIBLIOGRAPHY

- BODANSKY, M. 1923. *Journ. Biol. Chem.*, lvi, 387.
CAMPBELL, W. R. AND E. J. MALTRY. 1928. *Journ. Clin. Invest.*, vi, 303.
CARPENTER, T. M. AND E. L. FOX. 1930a. *Journ. Nutrition*, ii, 375.
1930b. *Journ. Nutrition*, ii, 389.
CARPENTER, T. M. AND E. L. FOX. 1930. *Arbeitsphysiol.*, iv, 545.
CARPENTER, T. M. AND R. C. LEE. 1932a. *This Journal*, cii, 635.
1932b. *This Journal*, cii, 646.
CORI, C. F. 1925. *Proc. Soc. Exper. Biol. and Med.*, xxiii, 290.
CORLEY, R. C. 1928. *Journ. Biol. Chem.*, lxxvi, 31.
DEUEL, H. J., JR. 1927. *Journ. Biol. Chem.*, lxxv, 367.
WIERZUCHOWSKI, M. AND M. LANIEWSKI. 1931. *Biochem. Zeitschr.*, ccxxx, 173.

THE DIURNAL CYCLE IN THE LIVER

I. PERIODICITY OF THE CYCLE, WITH ANALYSIS OF CHEMICAL CONSTITUENTS INVOLVED

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Forsgren, in 1928, observed in the livers of rabbits a cyclic activity of the glycogen-forming and the bile-secreting functions. Using a microchemical technic, he learned that the secretion of bile alternated with the deposition of glycogen and that these two functions were not regulated merely by nutrition, but were probably correlated in some way with a rhythmic activity in the digestive organs. During the night there was a maximal deposition of glycogen and a minimal formation of bile, and during the day the reverse condition prevailed. Even when there was a continuous supply of food in the stomach there were clear signs of periodicity in the liver, so that the food factor alone did not determine the glycogen content. Forsgren, in 1929, designated these two alternating functions as the assimilatory and the secretory phases of the cycle.

Agren, Wilander and Jorpes (1931) described cyclic changes in the glycogen content of the livers of rabbits, rats, and mice, largely independent of food intake. Glycogen accumulated in the liver during the night and disappeared to some extent the next morning. Similar conditions occurred even in fasting animals. These authors studied the cyclic variations of glycogen of the liver of mice and rats under continuous feeding and during fasting, but did not study changes which ensue following a restricted period of available food and water.

Two years ago one of us (Higgins) studied the changes in the weight of the liver which occurred during fasting, after a single period in which food and water were available, and under conditions in which food was constantly available. Chickens, rats and rabbits were studied at that time. When the ratio of the weight of the liver to the weight of the body was plotted against time after feeding, the curve derived was, in every instance, a bimodal one. When chickens were fed from 9:00 to 11:00 a.m., and food and water thereafter withdrawn, the first mode occurred at 3:00 p.m. when the weight of the liver in grams, for each 100 grams of body weight, had increased from 2.0 to 2.8. The second mode occurred at 1:00 a.m. and the low point occurred somewhat later in the rabbit than in the chicken,

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the first from 5:00 to 6:00 p.m. and the second from 12:00 midnight to 2:00 a.m. No attempts were made at that time to determine the chemical constituents which were involved in the changes in the weight of the liver. Recently, however, a study has been made of these cyclic changes in the weights of the livers of white rats after a single feeding period, and at the same time changes in water, glycogen, protein, and fat were followed by chemical analyses.

METHOD. White rats, from our colony, three to five months of age were trained to eat and drink from 9:00 to 11:00 a.m., for four days preceding the experiment. Food and water were removed from the cages each day at 11:00 a.m. On the day of the experiment twenty-five rats, which had been without food and water for the preceding twenty-two hours were weighed and killed, by severing the cervical vessels, at 9:00 a.m. The livers were removed, exsanguinated as far as possible, wiped dry and weighed. These animals of known body and liver weight were the control rats with which the experimental animals were compared, and chemical determinations were made of the water, glycogen, protein, and fat content of the liver of ten of them.

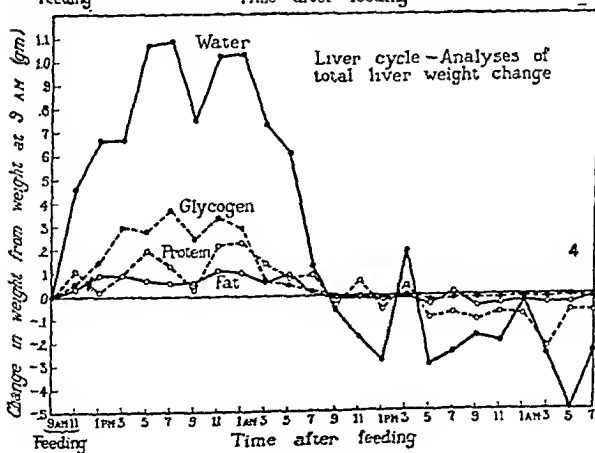
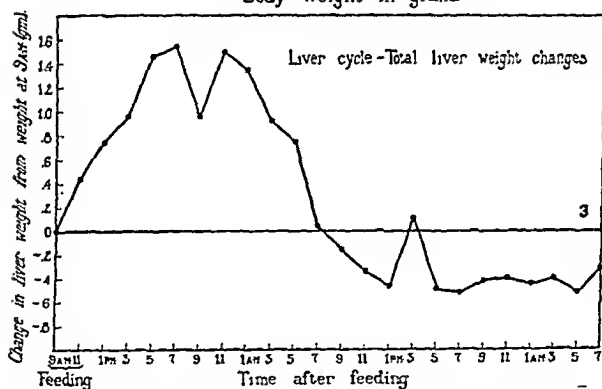
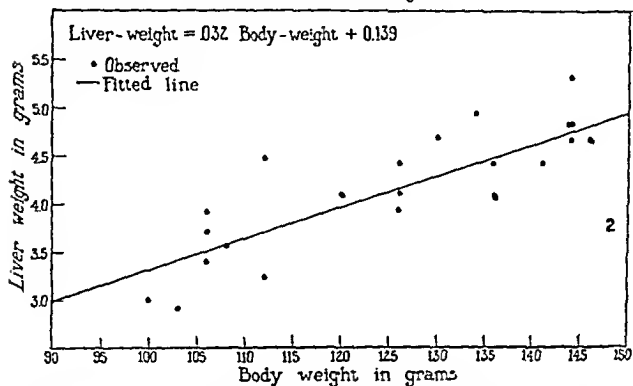
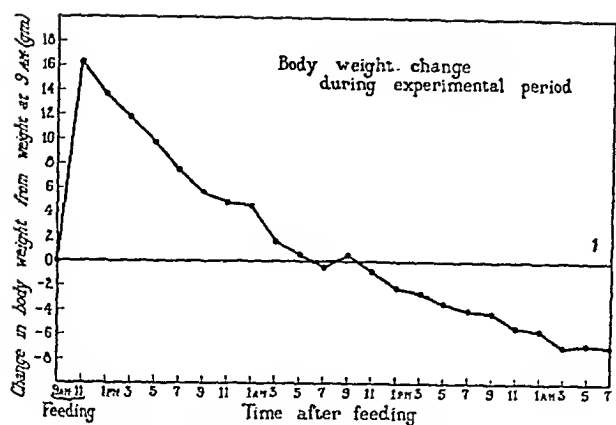
The experimental animals, which had also been fasted for twenty-two hours, were all weighed at 9:00 a.m. and food and water were placed in the cages from 9:00 until 11:00 a.m. At 11:00 a.m. all food and water containers were removed from the cages and beginning at this hour, and at every two-hour interval thereafter for forty-eight hours, six animals were weighed, and killed by severing the cervical vessels. The abdomen was quickly opened, the portion of liver for determination of glycogen was removed and dropped immediately into a freezing mixture of alcohol and carbon dioxide ice. These portions, usually of about 1 gram, were weighed, treated according to the method of Pflüger for glycogen, and the glucose formed was determined by the method of Folin. Other portions of the liver were removed similarly, weighed and analyzed for water, protein and fat. The same region of the liver was selected each time for each particular analysis. For the determinations of water, specimens of liver were dried at a temperature of 85° to 90°F. for a period of seven days, or until constant weight was reached. The loss in weight was determined and considered as water. For the protein analysis the Kjeldahl method for total nitrogen was used, and the value obtained was multiplied by the factor 6.25. The method used for the determination of total fat was essentially that of Bloor. Continuous alcoholic extraction of the samples was followed by the evaporation of the solvent and the extraction of the residue with petroleic ether. The ether soluble substance was dried, weighed, and considered as total fat.

OBSERVATIONS. The mean changes of the weights of the body of all the rats during the two day observation period were computed from their

weight at 9:00 a.m. (fig. 1). The animals gained, on the average, 16.2 grams during the feeding period from 9:00 to 11:00 a.m. A fall in weight was evident immediately thereafter, and this continued without interruption until the end of the experiment. It is of interest that at the end of the first twenty-four hours the mean body weight had returned to about the level recorded at the beginning of the feeding period.

Since the weight of the body was continuously decreasing, it was obviously inexact to express the changes in the liver in terms of a ratio of the weight of the liver to the weight of the body. It appeared preferable to express these changes in terms of the amounts added or lost directly, rather than in terms of a ratio. In order to obtain the change in weight that had taken place at any given hour, since 9:00 a.m. which was taken as the base, it was necessary to have an estimate of the weight of the liver of every experimental animal at 9:00 a.m. To obtain this estimate, we used the weight of the body and the weight of the liver of the twenty-five control rats which had been killed at 9:00 a.m. The weight of the liver was correlated with the weight of the body, and the correlation was found to be linear (fig. 2). A straight line was fitted by least squares to the twenty-five pairs of observations. From the regression equation so obtained ($L.W. = 0.032 (B.W.) + 0.139$, in which $L.W.$ is weight of liver and $B.W.$ is weight of body) the weight of the liver of each experimental animal at 9:00 a.m. was estimated from its known body weight at 9:00 a.m. Subsequently, the weight of the liver of each experimental animal was determined at the time it was killed, and the difference between the observed weight and that estimated at 9:00 a.m. gave the change in the weight of the liver for that animal. The mean change in the weight of the liver of six animals at each two-hour interval was used as the basis of our calculations.

The experimental findings of the mean changes in the weight of the liver at each two hour interval from the estimated weight at 9:00 a.m., together with their probable errors, have been assembled (table 1). Plus changes denote increases over the weight at 9:00 a.m. while minus changes are decreases from the weight at that time. The curve of these changes in weight (fig. 3) for the first twenty-four hours is definitely bimodal, but is less obviously so in the second twenty-four hour period. The first mode, which indicated the greatest increase in the weight of the liver, occurred at 7:00 p.m. although it was almost as high at 5:00 p.m. The second mode occurred at 11:00 p.m. when the change in weight of the liver recorded was almost as great as that found at 7:00 p.m. At 9:00 p.m., which marked the time of the low point between the two modes, the increase of weight had dropped to 0.968 gram from its previous high point of 1.537 grams. This figure was based on the weights of the livers of nine animals killed at that time.



Figs. 1 to 4

When the value of the mean change at the low point of 9:00 p.m. was compared with those of the modes on either side of it, that is, at 7:00 and 11:00 p.m., the difference in each case was found to be significant statistically. In the first instance, the difference is five times its probable error, and in the second, four times its probable error. Thus the low point 9:00 p.m. reflects a real loss in the weight of the liver from its previous high value, a loss probably associated with physiologic changes correlated with metabolism.

During the second twenty-four hour period the curve of changes in weight of the liver was also bimodal, although the second mode is less clearly defined than the first. All recorded changes, except that at 3:00 p.m., are below the base line and indicate a loss in the weight of the organ from its value at the beginning of the experiment. The mode at 3:00 p.m. is significant statistically, for its probable error is small and the difference between the mean at 1:00 p.m. and at 3:00 p.m. is several times its probable error. The second mode probably occurred at 1:00 a.m., for the mean change in weight of the six animals killed at that time showed a negative change of -0.435 ± 0.168 gram, wherein the probable error was large.

The actual changes which occurred in the water; glycogen, protein and fat content of the liver during the experiment were computed on the same principle as that used in determining the total changes in hepatic weight. The mean percentage content of these constituents of the liver were determined for ten control rats at 9:00 a.m. after a twenty-two hour fast. The following are the percentages found: water 68.7, glycogen 0.82, protein 24.5, and fat 6.9. By multiplying the weight of the liver at 9:00 a.m. by the respective percentage determinations, the total grams of water, glycogen, protein and fat in the liver at 9:00 a.m. were estimated for each experimental animal. At each two-hour interval for the ensuing two day period corresponding percentages were determined, and from these data and the corresponding weights of the liver the amount of each constituent present at that particular interval was computed. The amount estimated at 9:00 a.m. deducted from the amount determined at the time the animals

Fig. 1. Curve of change of body weight in a series of rats from 9:00 a.m. until 7:00 a.m., two days later. Food and water were withdrawn at 11:00 a.m. the first day.

Fig. 2. Diagram of the weights of liver and weights of body of control rats from which formula for liver weight was derived.

Fig. 3. Curve of change in weight of liver following a single two-hour feeding period, from 9:00 a.m. until 7:00 a.m., two days later.

Fig. 4. Curve of the changes in water, glycogen, protein and fat following a single two-hour feeding period, from 9:00 a.m. until 7:00 a.m. two days later.

were killed gave the change recorded. It is obvious that the calculations do not describe interchanges which most certainly go on continuously in the liver, but give only those net changes which occurred between the intervals.

The mean, with its probable error, and the standard deviations, of the percentage determinations in the chemical analyses of each of the four constituents is given for each interval of the experiment (table 2). During the experiment the percentage of water was relatively constant, ranging from 71.4 per cent at 11:00 a.m. immediately after drinking, to 66.7 per cent at the end of the experiment. The average percentage of water for the 148 animals studied was 69.44. During the first twenty-four hours the glycogen percentage ranged from 0.82 at 9:00 a.m. to 6.510 at 7:00 p.m.,

TABLE I
Summary of changes in weight of liver: forty-six hours

FIRST TWENTY-FOUR HOURS			SECOND TWENTY-FOUR HOURS		
Time		Change in weight from 9 a.m.	Time		Change in weight from 9 a.m.
Actual	Elapsed		Actual	Elapsed	
	hours	grams		hours	grams
11 a.m.	2	0.449±0.094	9 a.m.	24	-0.157±0.061
1 p.m.	4	0.745±0.139	11 a.m.	26	-0.335±0.099
3 p.m.	6	0.960±0.834	1 p.m.	28	-0.461±0.099
5 p.m.	8	1.474±0.116	3 p.m.	30	+0.108±0.080
7 p.m.	10	1.537±0.053	5 p.m.	32	-0.495±0.094
9 p.m.	12	0.968±0.077	7 p.m.	34	-0.503±0.052
11 p.m.	14	1.498±0.170	9 p.m.	36	-0.416±0.077
1 a.m.	16	1.353±0.079	11 p.m.	38	-0.414±0.033
3 a.m.	18	0.924±0.130	1 a.m.	40	-0.435±0.168
5 a.m.	20	0.750±0.087	3 a.m.	42	-0.411±0.087
7 a.m.	22	0.056±0.090	5 a.m.	44	-0.517±0.075
			7 a.m.	46	-0.324±0.072

eight hours after feeding; a second peak of 5.720 occurred at 11:00 p.m., after which a gradual decline continued until 7:00 the following morning when a glycogen percentage of 0.235 was determined. During the second day the percentage of glycogen varied greatly, the mean for the twenty-four hours being 0.500. Determinations of fat content were remarkably constant during the entire experiment. Percentages ranged between 6.11 and 7.99, the average for the entire series being 6.850. Determinations of protein values varied during the first day from 24.66 to 20.90, the average being 22.96 per cent. The lowest protein percentage occurred at the time of the peak of glycogen, 7:00 p.m. During the second day the percentage of protein was higher and ranged from 24.66 to 30.09, the average being 25.97.

The actual changes in the amount of water and glycogen present in the liver at the time of killing from that estimated at 9:00 a.m. is shown (table 3). The curve of these changes is also included (fig. 4). It is clear that the change in the water content of the liver was the greatest of

TABLE 2
Percentage constituents of liver at various hours after feeding

TIME	WATER		GLYCOGEN		FAT		PROTEIN	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
First twenty-four hours								
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
9 a.m.	68.775±0.366	2.10	0.821±0.082	0.47	6.990±0.225	1.00	24.509±0.470	2.70
11 a.m.	71.438±0.138	0.50	1.920±0.105	0.38	6.207±0.082	0.21	24.668±0.427	1.55
1 p.m.	70.938±0.289	1.05	3.578±0.187	0.62	7.290±0.339	0.87	22.680±0.338	1.12
3 p.m.	68.162±0.218	0.79	6.215±0.259	0.94	6.513±0.152	0.39	22.375±0.449	1.63
5 p.m.	69.145±0.245	0.89	5.155±0.050	0.18	6.460±0.078	0.20	22.112±0.333	1.21
7 p.m.	68.620±0.101	0.26	6.507±0.280	0.72	6.990±0.109	0.28	20.903±0.051	0.13
9 p.m.	69.398±0.232	1.03	5.306±0.202	0.90	6.672±0.179	0.65	21.299±0.459	2.04
11 p.m.	68.509±0.531	2.36	5.723±0.413	1.50	7.048±0.094	0.34	21.658±0.380	1.69
1 a.m.	69.943±0.263	1.17	5.460±0.410	1.49	6.875±0.140	0.51	22.744±0.277	1.23
3 a.m.	70.054±0.311	1.03	2.160±0.343	0.72	7.120±0.176	0.37	23.086±0.510	1.69
5 a.m.	69.798±0.223	0.74	1.445±0.329	0.69	7.990±0.005	0.01	23.650±0.509	1.51
7 a.m.	69.708±0.404	1.34	0.235±0.010	0.02	6.780±0.210	0.44	25.935±0.327	0.97
Second twenty-four hours								
9 a.m.	69.328±0.284	1.03	0.663±0.124	0.45	7.247±0.314	1.14	25.313±0.774	2.81
11 a.m.	69.332±0.039	0.14	0.522±0.036	0.12	7.328±0.306	1.11	30.095±1.809	6.57
1 p.m.	69.128±0.165	0.60	0.468±0.072	0.26	7.020±0.338	1.12	26.573±1.093	3.97
3 p.m.	69.868±0.253	0.92	0.610±0.110	0.40	6.363±0.121	0.44	25.552±0.710	2.58
5 p.m.	68.923±0.121	0.44	0.447±0.069	0.25	6.587±0.135	0.49	25.427±0.606	2.20
7 p.m.	70.565±0.509	1.85	0.455±0.184	0.67	7.894±0.609	2.02	26.082±0.567	2.06
9 p.m.	70.297±0.628	2.28	0.488±0.042	0.14	6.110±0.085	0.31	24.665±0.300	1.09
11 p.m.	70.142±0.438	1.59	0.425±0.030	0.11	6.392±0.127	0.42	25.343±0.363	1.32
1 a.m.	70.188±0.377	1.25	0.578±0.030	0.10	6.794±0.166	0.55	25.514±0.250	0.83
3 a.m.	68.730±0.154	0.51	0.404±0.033	0.11	6.310±0.238	0.79	25.824±0.718	2.38
5 a.m.	69.220±0.235	0.78	0.710±0.148	0.49	6.632±0.181	0.60	26.218±0.489	1.45
7 a.m.	66.718±1.023	3.39	0.266±0.039	0.13	6.830±0.299	0.99	25.074±0.281	0.93

all changes which occurred, and its bimodal curve rather closely follows the curve of the total weight change (fig. 3). The first mode occurred at 7:00 p.m., when the water increase above that present at 9:00 a.m. was 70 per cent of the total weight increase. At 9:00 a.m. on the second day

the water content was slightly below that recorded at the beginning of the experiment, a loss of 0.06 gram having occurred. However, the curve for the second day appears also bimodal, although the changes during this period are not as marked as those which occurred during the first twenty-four hours.

TABLE 3
Summary of composite data

TIME		CHANGE IN WEIGHT FROM 9 A.M.	
Actual	Elapsed	Water	Glycogen
First twenty-four hours			
	<i>hours</i>	<i>grams</i>	<i>grams</i>
11 a.m.	2	0.463±0.069	0.0560±0.0052
1 p.m.	4	0.669±0.107	0.1530±0.0109
3 p.m.	6	0.668±0.168	0.3010±0.0330
5 p.m.	8	1.076±0.085	0.2790±0.0072
7 p.m.	10	1.084±0.043	0.3710±0.0206
9 p.m.	12	0.752±0.067	0.2470±0.0148
11 p.m.	14	1.024±0.011	0.3330±0.0441
1 a.m.	16	1.036±0.007	0.2940±0.0242
3 a.m.	18	0.733±0.193	0.0630±0.0215
5 a.m.	20	0.609±0.054	0.5250±0.0253
7 a.m.	22	0.137±0.039	0.0240±0.0029
Second twenty-four hours			
9 a.m.	24	-0.0603±0.016	-0.0040±0.0055
11 a.m.	26	-0.1801±0.072	-0.0120±0.0010
1 p.m.	28	-0.2888±0.066	-0.0200±0.0173
3 p.m.	30	+0.1938±0.077	-0.0070±0.0055
5 p.m.	32	-0.3030±0.063	-0.0210±0.0019
7 p.m.	34	-0.2490±0.033	-0.0176±0.0036
9 p.m.	36	-0.1801±0.078	-0.0158±0.0017
11 p.m.	38	-0.2048±0.017	-0.0166±0.0010
1 a.m.	40	-0.0388±0.106	-0.0110±0.0033
3 a.m.	42	-0.2614±0.057	-0.0170±0.0009
5 a.m.	44	-0.5070±0.148	-0.0078±0.0051
7 a.m.	46	-0.2524±0.066	-0.0234±0.0009

The curve of the glycogen changes follows fairly well those of the total liver and water, in that the modes occurred at 7:00 p.m., 11:00 p.m. and the low point at 9:00 p.m. The dip in the curve seen at 5:00 p.m. is not significant statistically.

During the second day the glycogen changes were all negative, that is, there was some loss since the beginning of the experiment and their curve is practically a straight line, the livers containing but little glycogen.

Glycogen in the liver appears to be dependent on an available supply of food or water.

The protein changes were also cyclic in their distribution throughout the first twenty-four hours, although the modes did not exactly conform to those of the water and glycogen curves (fig. 4). The first mode occurred at 5:00 p.m. when an increase of 0.201 ± 0.030 gram had occurred since 9:00 a.m. and the second mode occurred at 1:00 p.m. when an increase of 0.230 ± 0.011 gram was recorded. The low point between the two was at 9:00 p.m., agreeing in this respect with both the water and glycogen changes. During the second day the determinations showed both increases and decreases from the base level at 9:00 p.m., but a cyclic distribution of these changes in protein did not appear.

COMMENT AND SUMMARY

A study of some of the changes which occurred in the liver of the white rat after a two hour feeding and drinking period is reported. These changes include the actual weight of the liver, as well as the increases or decreases in the amount of water, glycogen, protein and fat for a two-day period after a single feeding.

By means of a formula, determined from statistical data assembled from twenty-five rats, at 9:00 a.m., after a twenty-two hour fast, we were able to estimate the weight of the liver of each experimental rat at that time. During the interval from 9:00 to 11:00 a.m. of the first day of the experiment all rats had access to food and drink. Forced feeding was not attempted, but any animal not freely partaking of food was eliminated from the study. Six animals were killed at every two-hour period and nine at crucial points during the cycle as at 5:00, 7:00, 9:00 and 11:00 p.m. of the first day.

The curve of the changes in the weight of the liver is definitely bimodal. The increase in the weight of the organ continued uninterruptedly until 7:00 p.m.; a significant decrease was encountered at 9:00 p.m. This was transient, however, for the change in the mean weight of the liver at 11:00 p.m. was almost as high as that found at 7:00 p.m. From 11:00 p.m. on a decrease in the total weight was recorded so that at 7:00 the following morning the mean weight of the livers of the six animals killed was essentially that of twenty-four hours before.

We have observed this same bimodal cycle in all animals studied. It occurs in chickens, rabbits and guinea pigs. It may not occur always at the same hour in all animals, but our study has led us to conclude that there are two peaks to the curve of the changes in the weight of the liver during the digestive phase which follows feeding. We have not attempted an explanation to account for the transient decrease in the weight of the liver which occurred at 9:00 p.m. in our series of rats, but it is probably

correlated with the physiologic processes within the liver or perhaps with a rhythmic activity in the gastro-intestinal tract.

During the second day the changes in the weight of the liver were essentially all negative in the sense that the weights were below those estimated at 9:00 a.m. on the first day. A significant rise was encountered at 3:00 p.m. although an immediate decrease recorded at 5:00 p.m. was essentially equal to that recorded at 1:00 p.m. Thus the mode at 3:00 p.m. was comparable, except in extent, to that at 5:00 or 7:00 p.m. the first day. A second mode in the curve of the change in the total weight of the liver was not encountered during the second twenty-four hour period.

It is clear from a study of the curves of the changes in the four constituents of the liver that water, glycogen and to a less extent protein follow rather definitely the curve of the changes in the total weight of the liver. The first mode occurred at 5:00 or 7:00 p.m. and the second occurred at 11:00 p.m. or at 1:00 a.m.; the low point in each case occurred at 9:00 p.m. just as in the curve of the changes in the total weight of the liver. There is little to be said concerning the change in curve for fat, except that it did not follow a bimodal plan such as the changes for water, glycogen and protein, but the increases which were encountered followed more nearly a straight line.

During the second day there did not appear to be any cyclic change, except perhaps in the water content. The marked increase in the water content of the liver at 3:00 p.m. explained the mode encountered at that hour in the curve of the total change in weight. A second mode occurred at 1:00 a.m. but it was considerably less than that at 3:00 p.m. There were practically no changes in the glycogen content during the second day and the determinations were slightly less than those recorded at 9:00 a.m. at the beginning of the experiment. There appeared to be no deposition of glycogen in the livers of these rats in the continued absence of food, even during the night. Furthermore, the determinations of fat and protein largely represented decreases from those recorded at the beginning of the experiment and showed no particular cyclic activity.

BIBLIOGRAPHY

- AGREN, G., O. WILANDER AND E. JORPES. 1931. *Biochem. Journ.*, xxv, 777.
FORSQREN, E. 1928. *Skand. Arch. f. Physiol.*, lii, 137.
1929. *Journ. Morphol.*, xlvii, 519.

ELECTROMYOGRAPHIC STUDIES OF THE GASTRO- INTESTINAL TRACT

I. THE CORRELATION BETWEEN MECHANICAL MOVEMENT AND CHANGES IN ELECTRICAL POTENTIAL DURING RHYTHMIC CONTRACTION OF THE INTESTINE

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Action currents from the intact musculature of the intestine do not follow the simple forms described in the textbooks as characteristic for skeletal muscle. When recorded they show themselves usually as irregular curves of varying form. Often the records of the electric potential will show a wave-like form which will be more or less regular for a time and in phase with the mechanical contractions of the muscle, but suddenly their appearance will change; the wave form will become irregular, and it will be hard to trace a relationship between the electrical and the mechanical waves.

Recourse to the literature will hardly help clarify matters. Many records of researches on the action potentials of striated and cardiac muscle have been published, and one can find a didactic description of the behavior of electric potentials in any work on the electrocardiogram. The action currents from smooth muscle, on the other hand, have received little attention. From the published work in this field, one obtains the general impression that in interpreting the electrograms one may apply to smooth muscle the same principles that are involved in the electrobiology of skeletal muscle and nerve. When we first undertook this investigation it was with the idea of employing these principles to interpret observed changes of potential in relation to the mechanical movements. Our original plan was merely to continue with an improved technic the studies initiated by Alvarez and Mahoney. We had not proceeded far, however, before it became evident that the relationship between contraction and electric change obtaining in this domain required careful scrutiny, for it presented a number of features not to be expected on the basis of the usually accepted notions regarding action currents in muscle. This fundamental aspect of

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the problem was therefore made the first objective of our investigations. We believe we have made significant progress in that direction, and this report presents the results which we have obtained thus far.

APPARATUS AND EXPERIMENTAL TECHNIC. In order to register simultaneously mechanical contraction and variation in potential we adapted a form of enterograph used by Alvarez so that it should act also as a pair of receiving electrodes. It is pictured to scale in figure 1. The small clip, or serrefine, requires special description. It is made from steel piano wire, 0.018 inch in diameter, bent into the shape shown in the insert of figure 1. Unlike some observers, we have found it impossible to obtain satisfactory results with a polarizable metal electrode, and it was necessary to render the steel serrefine nonpolarizable. This was accomplished by first plating it with silver, and then chloridizing the silver electrolytically.² Before use, each pair of electrodes was tested for its nonpolarizable quality.

For most of the investigations reported here, rabbits were used. The animals were anesthetized with urethane or iso-amyl-ethyl barbiturate (amytal) and pithed following the technic described by Alvarez. A median line abdominal incision was made and the intestines were freely exposed.

Action currents may be recorded from the intestines with the animal in the air, or in a warm bath of salt solution but both these arrangements have practical objections. In the air, the animal must be kept warm artificially and the intestine tends to dry, thereby changing the circuit resistance while a record is being taken. In the bath of salt solution the surrounding fluid tends to short-circuit the electrodes and thus to diminish sensitivity. Such a bath tends also to conduct to the electrodes extraneous currents developed here and there in the body. For these reasons an arrangement was made to permit the animal to rest in a warm bath of water, while the intestines floated in inert mineral oil.

It was soon found that any metal coming in contact with the solution of salt would produce electric currents and we therefore had recourse to a

² For any who may wish to duplicate this part of the apparatus there are a few practical precautions which must be taken: 1. The steel wire serrefine must be scrupulously clean of fatty covering, and therefore before silver plating it should be boiled in concentrated alkali. For convenience in handling the serrefine during the plating process a short piece of the same type of wire is soldered to the loop. This serves also to lead in the current. 2. Pure silver must be used as the anode in the silver plating process, and for the plating bath, a solution is made up as follows: AgCN, 36 grams per liter, KCN, 52 grams per liter, 1 drop CS₂. 3. The tendency is to silverplate too slowly which gives a rough surface; we use a current density of 10 to 15 milliamperes for each electrode, and plate for three to four hours. 4. In the chloridizing process, the electrode is the anode. A N/2 NaCl or HCl solution is used for the bath, and a low current density, about 1 to 2 milliamperes for each electrode, is applied for about half an hour. 5. In chloridizing, the juncture of the serrefine and the lead-in wire must not be allowed to get into the solution lest the joint forms an electric cell with resultant disintegration.

wooden tank water proofed with wax and varnish. The thermostat and heating elements were all enclosed in glass. The tank was filled to a convenient height with physiologic sodium chloride solution kept at 37°C. and the opened animal was placed into it, its head resting on a canvas support out of the water. A wooden frame about 30 cm. square was then inserted in the tank, and the intestines were allowed to float up into it. The electrodes were now attached to the moistened intestine, and then a quantity of mineral oil sufficient to provide a layer 2 cm. in depth, was poured into the frame to surround the intestines. The tissue to be ex-

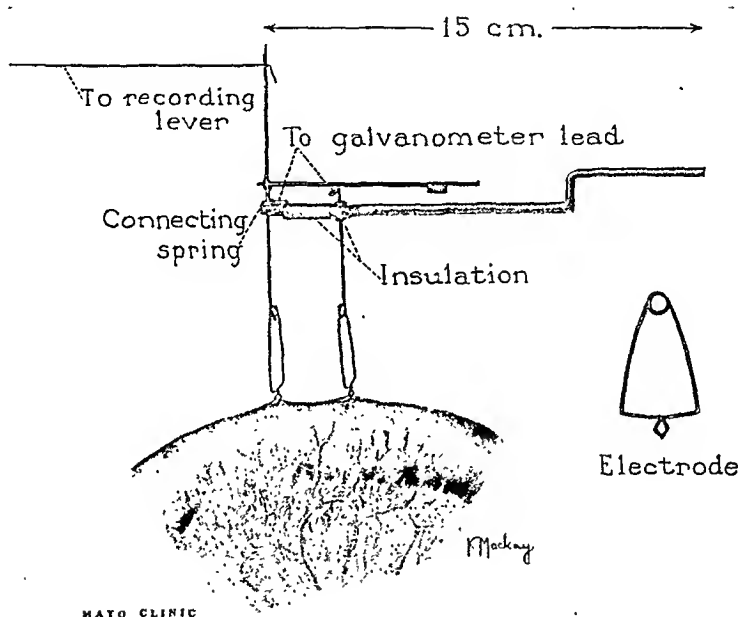


Fig. 1. The apparatus for simultaneous electric and mechanical recording. The electrodes are insulated at the points marked. The connecting spring is made of light copper and is inserted to insure conduction across the joint. The insert shows the serrefine-electrode in detail.

amined was now in an electrically non-conducting medium. The remainder of the body was in a congenial environment which was electrically conducting, a point important for certain of the experiments to be described later. In this state the animal could be maintained for an entire day's observation. At the end of the experiment, the oil was recovered by siphoning.

The changes of electrical potential were recorded by means of a Cambridge string galvanometer, the shadow of the string being photographed on moving bromide paper 12 inches in width. The movement of the intestine was recorded simultaneously on the same paper, by attaching the freely-

swinging electrode with a fine thread to an aluminum lever suspended in the lighted area in front of the camera slit.

RESULTS OF EXPERIMENTS. If a pair of nonpolarizable electrodes are attached to the rabbit's intestine and a simultaneous electrical and mechanical record is made, the results will in general appear as an irregular electric disturbance, with a mechanical record that seems in many instances to have little relation to it. In figure 2 is presented a sample of various records, selected at random from our series.³ The forms shown are diverse, and still other examples could be multiplied indefinitely. It must be remembered that the intestine commonly shows various types of activity such as pendular movements, tonus waves, and peristaltic rushes,

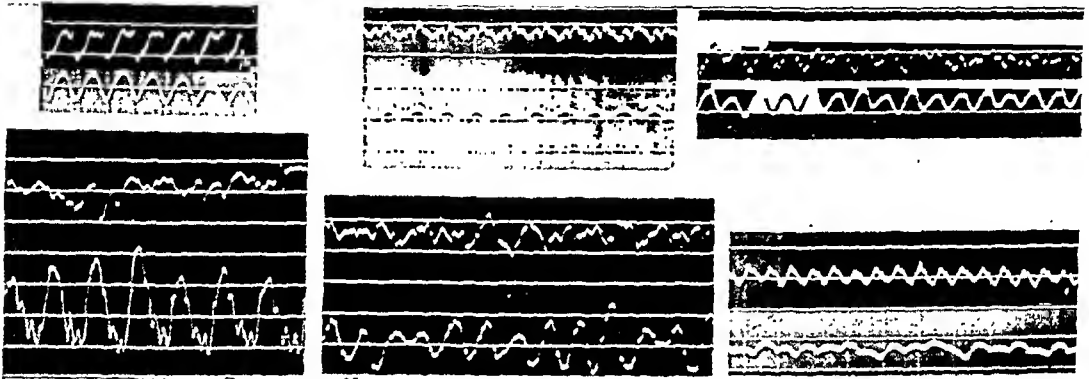


Fig. 2. A sample of various forms of simultaneous electric and mechanical records. The variety could be multiplied indefinitely. In each case the upper record is the electric and the lower is the mechanical. The interval between the time marks is five seconds.

and the rhythmic contractions have different rates in different parts of the intestine. It was reasonable to assume that the varying and irregular form of the electric record, so commonly obtained, was due to the fact that many muscular elements contract with imperfect synchronism and thus contribute many changes of potential to the record. If this were true, we could expect to simplify the record obtained by including smaller amounts of muscular tissue. For this reason we took a very small bite of the serosa with the serrefine-electrode, and attached the electrodes in close approximation to each other, that is, about 1 or 2 cm. apart. These conditions

³ The electrodes were always attached in a standard way. The one which is free to move back and forth was attached caudally to the fixed one, and the galvanometer was so wired to the electrodes that an upward deflection in the record indicates that the oral electrode has become relatively negative to the caudal one. In the mechanical record an upward deflection corresponds to contraction, and a downward to relaxation.

were adhered to in all the experiments to be described except when otherwise stated.

Doubtless the seeming anarchy of the variations of potential as usually seen is only apparent. If we could analyze completely the multifarious

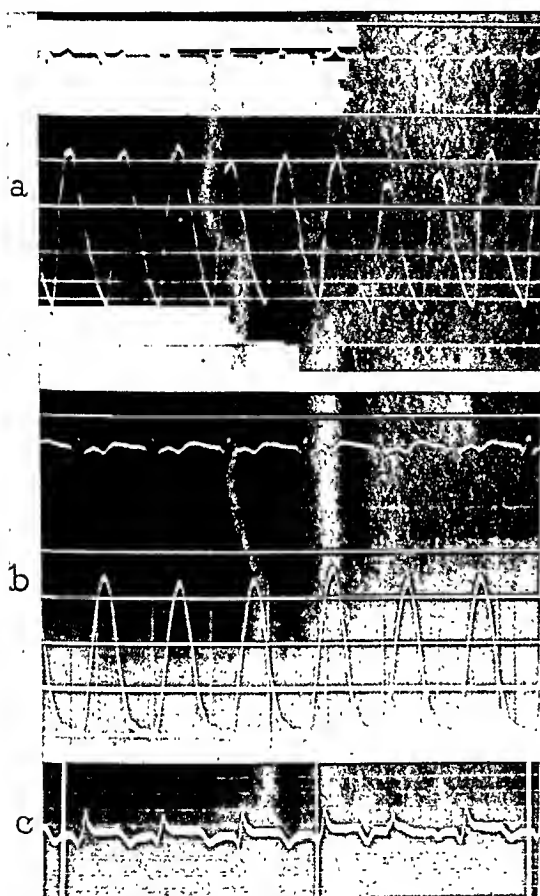


Fig. 3

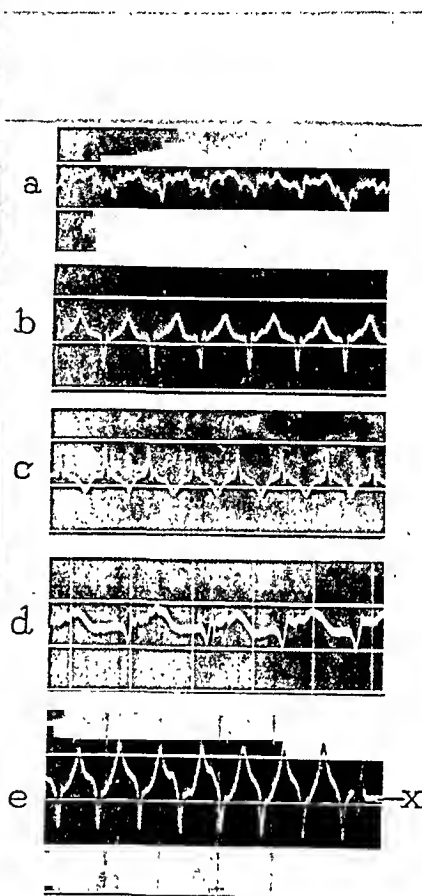


Fig. 4

Fig. 3. a. An example of a characteristic electric wave, with accompanying mechanical record. The upper is the electric, the lower the mechanical record. b. An example of an inverted wave (see text), obtained with bipolar attachment; below it is the simultaneous mechanical record. c. A bipolar record from an isolated segment of a dog's intestine, for comparison (Puestow).

Fig. 4. a. A unipolar record from the oral electrode. b. A unipolar record from the caudal electrode. c. A bipolar record from the same electrodes made in the standard way. The bipolar record is the algebraic difference of the oral and caudal unipolar records. d. A characteristic wave enlarged to show rapid oscillation during the contraction phase. e. To show the presence of a positive variation during the course of rhythmic contraction. The line at the right, *x*, is the level of zero potential relative to the bath. A deflection below the line indicates a positive change in potential.

activities of the intestine, the record of electric potentials would probably also be clarified. To simplify the analysis somewhat we are reporting only on records obtained from intestine which was executing regular rhythmic contractions. Under these circumstances more regular records are obtained, but they are still far from uniform. In many cases the records are complicated because the electrodes pick up currents that arise in movements of respiration. In what follows we shall describe in some detail a particular form of wave, which we have been able to analyze more successfully than any other. In doing so we do not wish to convey the impression that we have made a complete analysis of the electromyographic record. Much more work must yet be done before this can be effected. The type of electrogram which we here describe appears about once in every ten records taken at random along the intestine, and when seen in its characteristic form it has a definite relationship to the mechanical record.

Both the electric and mechanical records show clearly-defined wave forms, which have the same rate (fig. 3a). The beginning of the wave of contraction, however, does not coincide in time exactly with the beginning of the corresponding electrical wave, but occurs somewhat later. The interval varies in our records and it is most easily accounted for as lag due to the inertia of the mechanical system of recorders. If we align the beginnings of two corresponding waves, a simple relationship over their whole course is apparent. The contraction phase of the mechanical record corresponds exactly to the first half of the electric wave, and the relaxation phase exactly to the second half. These relations are shown more clearly in figure 5, which outlines what we believe to be the correlation of the electric and mechanical phases of rhythmic contraction. The electric wave is seen to consist of two parts. The first half of the wave is a deflection in the upward direction, the second half in the downward direction. The second half is a mirror image of the first. The first part corresponds in time to contraction, the second to relaxation; in other words, the observed electric changes during relaxation are the same as those during contraction, but they occur in the reverse order.

Our accumulated records are illustrative of another striking feature. There are a large number in which the same form of wave appears in the same time relation to the mechanical record, but the entire wave is inverted, that is, the initial deflection in its varying amplitude is in the downward or positive direction (fig. 3b). The record is such as one would expect to obtain by reversing the leads to the galvanometer. It is to be remembered that for all these records, not only those that give the upright type of wave but also those that give the inverted type, the fixed and the movable electrodes bear the same relationship to the oral-caudal direction of the intestine, and that the galvanometer string always deflects upward when the oral attachment is becoming negative relative to the caudal.

Why, then, does the oral attachment sometimes become relatively negative, and sometimes relatively positive to the caudal?

For a while, the explanation was sought in some condition of the intestine, or in the part of the intestine from which the record was obtained. But the question was resolved very simply when a different type of experiment was performed. Instead of connecting the galvanometer to both electrodes that are attached to the intestine, we attached only one; the other was connected with a neutral or standard nonpolarizable electrode, immersed in the bath in a corner of the tank at a distance from the body of the animal. The galvanometer was connected in such a way that an upward deflection indicated that the electrode on the intestine was negative relative to the inactive standard electrode.

When unipolar records were thus taken, the initial deflection was always directed upward, that is, in the negative direction. It is at once clear how

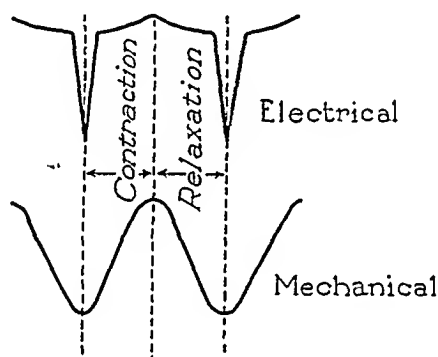


Fig. 5. Diagrammatic illustration of the relationship of the phases of the electric wave to those of the mechanical contraction, when the beginnings of both waves are made to coincide.

an inversion of the wave occurred in the bipolar records. At each electrode the typical wave of the upright type was produced. Since the electrodes were reasonably close, the mechanical contraction was generally in the same phase at both points, and the electric waves were generally identical except for amplitude. If it happened that the wave at the caudal electrode had the larger amplitude the bipolar record would be reversed, for the galvanometer registers the difference in potential between the leads, and it is to be recalled that under the arrangements of the experiments, it was the caudal electrode of the bipolar records that replaced the standard of the unipolar.

That this is the explanation of the appearance of the inverted form of the wave is easily demonstrated experimentally. In figure 4 a, b, and c are shown, first, two unipolar records, one from the oral, the other from the caudal electrode; both are typical wave forms in the normal direction, al-

though that from the oral electrode is not so sharply defined on account of disturbances due to respiratory movements. When the electrodes are attached to these same points for a bipolar record in the regular way, the algebraic difference of these is obtained, and since the caudal wave is of greater amplitude it predominates and the resultant wave is in the reversed direction.

Up to this point we have considered the characteristic electric wave, obtained during rhythmic contraction, in its general outline. We shall now take up several details:

1. The part of the typical wave corresponding to contraction, that is, the first half of the wave, is further divisible descriptively into two elements, quite sharply defined (fig. 5). The first of these is a rapid upward deflection occupying about one-fifth of the entire contraction period, and ending abruptly. This is followed by a deflection, indicating a stationary, or slowly rising negative potential occupying the rest, that is, the last four-fifths of the contraction period. The latter is completed by a short rapid rise just before the beginning of relaxation. The relaxation phase, being the mirror-image of that corresponding to contraction, consists of two similar parts occurring in reverse order. It is to be presumed that the two component parts of each half wave of electric potential so strikingly different in appearance, represent different processes in the physiology of muscular activity. These findings raise the question of what may constitute these physiologic differences.

2. The contraction phase of the electric record contains one detailed character not present in that of relaxation. Just after the sharp rise at the beginning of contraction, there is a rapid oscillation of the string at the rate of about 10 per second. It can be seen clearly in the enlarged figure 4 d and can be made out in the waves of figure 3. These oscillations are not always observed, but are seen so frequently that we are inclined to believe that if they are absent, it is because of a lack of sufficient sensitivity in the recording devices. We have seen the same sort of rapid oscillation of the string, regularly, during tetanic contraction of the intestine, when studying peristaltic rushes. Perhaps, during rhythmic contractions, too, they represent a tetanic-like state of the muscle as a part of the contraction process.

3. The next feature to be considered is not evident in all records. In fact, it was not observed until late in our investigations, and only after special consideration did we decide that it forms a definitive part of the potential wave. We refer to the appearance, at times, of a sharp positive deflection just when one wave ends, and the next begins. This is shown in figure 4 e, in which the level of zero potential relative to the standard electrode of the bath is shown by the line at the right. When the shadow of the string is below this line, the intestine at the point of attachment of the

electrode is at a positive potential, when above, at a negative potential relative to the standard electrode. It is to be observed that at the end of a wave there is a rapid deflection of the string below the zero line (positive) and an equally rapid return merging into the next wave. The sharp downward deflection referred to is, then, due to a relatively positive potential of the intestine.

Considering the conditions of the experiment, the foregoing observation is surprising. According to the almost universally stated theories, action currents in muscle originate in a wave of negativity that accompanies excitation. According to this conception, a positive deflection in the action curve can be produced only when a bipolar record is made, and in that case the positive deflection is due to an active negativity in the distal electrode. But we were working with a unipolar arrangement. It is difficult to frame any hypothesis by which the standard electrode in the bath could be affected negatively by the contraction of the intestinal muscle far removed from it. But, more important still, it is well nigh impossible that it should be so affected, in such precise relationship to the phases of the wave of potential from the muscle, for these phases do not coincide in time for waves from different parts of the intestine. A negative pulse at the standard electrode, coördinated to the phases of one wave, would necessarily be out of phase with another. We seem, therefore, to be driven to the conclusion that the origin of the downward throw of the string referred to, is produced by a positive potential at the site of the active intestine. For such a phenomenon there is no place in the negativity hypothesis usually advanced to explain action currents from muscle and nerve. Similar observations have been made for striated muscle by Craib.

4. Another point is noteworthy because of its contrast with what is seen in the usual action-current tracings. We have shown that the wave of potential during relaxation is quite as marked in its definitive character as that during contraction. Such is not the case for action currents from striated muscle or nerve ordinarily. For instance, the entire P-Q-R-S-T wave of the electrocardiogram is completed during systole; no changes in electric potential occur during diastole. The same is essentially true for skeletal muscle; the electric changes observed during relaxation are merely due to the passing off of the previously established negative potential. This is not the case in our observations; there was a definite character to the potential wave during relaxation (the same indeed as during contraction), it has a point-to-point correspondence with the mechanical record of relaxation, and its duration in time is exactly the same. In view of the fact that it is so unusual to observe action-current changes during relaxation of muscle, we hesitate to advance this observation as contradictory of the general finding.

It is conceivable that what we have observed in these experiments is

not strictly comparable with the ordinary action currents. Also it may be that the lengthening of the muscle is not always due entirely to relaxation, but is due partly at least to a pull resulting from contraction of the muscle in neighboring segments of the intestine. It is hard to see how such stretching could produce the particular form of wave observed, but the possibility should be kept in mind. It would not be surprising, however, to find indications of active potential changes accompanying relaxation in smooth muscle because this tissue is always in a state of tonus between complete relaxation and complete contraction. Since relaxation as well as contraction can be produced by stimulation of the appropriate nerve it may be looked on perhaps as an active process at least under some circumstances.

The wave of electric potential, which we have described for rhythmic contractions of the rabbit's intestine, does not appear to be limited to that animal. We have found evidence of its presence in some experiments on the dog and cat which we hope to report later. Puestow, working with segments of the dog's intestine transplanted to the abdominal wall obtained action currents which we would interpret as corresponding to the inverted type of wave in a "bipolar record" obtained in the rabbit. For comparison an example from Puestow's records is included in figure 3 c. Electrograms obtained by Castleton also of isolated dog's intestine, which he has kindly permitted us to examine, corroborate this impression.

SUMMARY

Simultaneous electric and mechanical records from the intact intestine of the anesthetized rabbit were made. Bipolar and unipolar records were studied. The unipolar is fundamental; the bipolar represents the algebraic difference between the unipolar potential changes at the two electrodes. During rhythmic contraction of the intestine, a characteristic type of wave of electric potential can be observed which has a definite relation to the mechanical contraction. The electric wave has a symmetric form; the first half occurs during contraction and the second half during relaxation. As a part of the contraction phase there are often a number of small rapid oscillations similar to those observed during tetanus. Unipolar records show that a positive variation sometimes occurs during the course of the contraction process.

BIBLIOGRAPHY

- ALVAREZ, W. C. 1928. The mechanics of the digestive tract. New York, Paul B. Hoeber, pp. 366-378.
- ALVAREZ, W. C. AND L. J. MAHONEY. 1922. *This Journal*, lvi, 476.
- CRAIB, W. H. 1930. Medical Research Council. Special report series, 147. London, His Majesty's Stationery Office.
- PUESTOW, C. B. 1932. *Arch. Surg.*, xxiv, 565.

EFFECTS OF CORTICO-ADRENAL EXTRACT ON GLYCOLYSIS IN VITRO¹

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Experiments recently carried out in this laboratory have shown that the adrenal cortex is intimately concerned with the regulation of carbohydrate metabolism in normal and in adrenalectomized animals (Britton and Silvette, 1931, 1932). Cortico-adrenal extracts which were made according to a slightly modified Swingle-Pfiffner technique increased hepatic and muscle glycogen and blood sugar, and concurrently decreased the blood lactates. Since the effects of the extract on carbohydrate metabolism in the intact animal were so well marked, it was thought that additional knowledge concerning the relationship of cortical extract to the metabolism of carbohydrates might successfully be acquired from experiments *in vitro*.

Earlier work on the effects of insulin and adrenalin on muscle-glucose systems has been mostly inconclusive (Cori, 1931). In the present investigation it was decided to work mainly with a blood-glucose-extract system, since the physical and chemical characteristics of blood lend themselves to accurate and easily controlled treatment.

To date we have made about 130 batches of cortico-adrenal extract (Swingle and Pfiffner, 1931), and have found the material effective on adrenalectomized and normal animals. The different extracts were not equally potent, however, so that experiments reported herein cannot always be quantitatively compared one with another unless the same extract happened to be employed in each case.

METHODS. Blood was withdrawn by syringe and needle from the hearts of normal, well-nourished dogs, and defibrinated by shaking in a flask with a number of glass beads and filtering through a wisp of cotton. Immediately after defibrination it was carefully mixed, measured out and used.

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² Porter Fellow of the American Physiological Society.

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Sufficient amounts of a solution of pure glucose in 0.8 per cent sodium chloride were added to bring the absolute amount of glucose present to between 10 and 15 mgm. (see tables). The cortico-adrenal extracts and also the 1:2,000,000 adrenalin control solution employed were made isotonic with sodium chloride before being added to the blood. Sufficient normal saline solution was then added to bring the contents of the tubes to 10 cc. The erythrocytes were thus suspended in a matrix of the same pH and osmotic pressure as that of whole blood.

Our extracts when quite fresh (immediately after having been Seitz-filtered) have been observed to have a pH slightly above 7 by the quinhydrone electrode. With age, however, the extracts seemed to grow more acid; extracts a week old commonly had a pH of 6.0 to 6.5, while still older ones have been as acid as pH 4.5 to 5.0. In these experiments fresh extracts have been used in most cases, although the following experiment showed that even relatively small quantities of defibrinated blood had sufficient buffer action to take care of relatively large amounts of an extract as acid as pH 4.52 (extract no. 122, two weeks after completion):

Tube 1: 1 cc. blood, 0.25 cc. extract, 3.75 cc. saline; pH 7.48.

Tube 2: 1 cc. blood, 1.50 cc. extract, 2.50 cc. saline; pH 7.44.

After careful mixing of the contents of the tubes, samples for glucose analysis were withdrawn by means of a 0.1 cc. Folin blood sugar pipette, care being taken that the same pipette was used throughout an experiment. The tubes containing the blood-glucose-extract mixture were placed in a specially constructed shaker-incubator maintained at a temperature of 38 to 39 degrees, in which they were shaken about 70 times a minute for a period of three hours. Intermediate and final samples for analysis were taken only after the contents of the tubes had been again cooled to room temperatures. Samples for lactic acid determinations were collected with similar precautions before and after incubation. Practically all experiments were run in pairs; furthermore all determinations were made in duplicate, i.e., the figures given in the tables represent the average of 2 to 4 readings.

Glucose was determined by the method of Folin and Malmros (1929), and lactic acid by the method of Friedemann, Cotonio and Shaffer (1927).

RESULTS. In order to determine the normal variation in the rate of glycolysis in a series of control tubes, a number of experiments were performed as follows:

Equal quantities of defibrinated blood from a normal animal were suspended in equal volumes of glucose-saline solution, and the tubes containing the blood-glucose mixture placed in the shaker-incubator for three hours. Initial and final blood glucose determinations were made. A typical control experiment is given below.

Forty per cent defibrinated blood, 60 per cent glucose-saline solution. Incubated 3 hours. Initial glucose concentrations, respectively, 154, 157, 155, 157, 156, 154 mgm. per cent. Final glucose concentrations, respectively, 130, 126, 126, 133, 128, 126 mgm. per cent. Per cent decrease in glucose over 3 hour period, respectively, 15.6, 19.7, 18.7, 15.3, 18.0, 18.1,—mean, 17.6.

In the above experiment, the greatest positive divergence was 11 per cent greater than the mean, and the greatest negative divergence was 13 per

TABLE 1
Effects of different amounts of cortico-adrenal extract on glycolysis in vitro

EXPERIMENT	BLOOD	EXTRACT	GLUCOSE		DECREASE IN GLUCOSE	CHANGE FROM CONTROL
			Before incubation	After incubation		
	per cent	per cent	mgm. per cent	mgm. per cent	per cent	per cent
18	15	0	126	117	7.1	
	15	5	120	109	9.2	+ 30
	15	10	123	98	16.3	+129
	15	20	120	115	4.2	-41
	15	30	126	121	4.0	-44
5	30	0	146	125	14.4	
	30	10	148	119	19.6	+36
10	30	0	135	120	11.1	
	30	5	139	114	18.0	+62
	30	10	139	122	12.2	+10
	30	30	142	130	8.5	-23
6	35	0	151	139	8.0	
	35	10	152	136	10.5	+31
21	60	0	144	107	25.9	
	60	2.5	142	87	38.8	+47
	60	10	142	118	20.3	-21
	60	30	142	125	12.0	-54
14	87	0	105	47	55.2	
	87	9	105	53	49.5	-10
	87	16	100	58	42.0	-24

Experiments 2, 8, 9, 11, 17, 19 and 20 resulted similarly; details omitted in the interests of conservation of space.

cent less than the mean. From table 1 it will be apparent that in no case in the extract-containing tubes was the increase in glycolysis less than 17 per cent more than that occurring in the control tubes, with the great majority of increases above the control rates over 30 per cent. Conversely,

in only one case in which the inhibitory effect of the extract is demonstrated is the decrease in the rate due to extract less than 20 per cent below the rates occurring in the control tubes.

The presence of cortico-adrenal extract within limits in the blood-glucose system induced a markedly higher rate of glycolysis than was observed in the control tubes (table 1). Large quantities of the extract (20-30 per cent concentration) nevertheless inhibited glucose disappearance very considerably. In this connection it is interesting to note that Ahlgren (quoted by Cori, 1931) found that the optimal concentration of insulin

TABLE 2
Effect of adrenalin and boiled cortico-adrenal extract on glycolysis in vitro

EXPERIMENT NUMBER	BLOOD	SUBSTANCE ADDED		GLUCOSE		DECREASE IN GLUCOSE	CHANGE FROM CONTROL
		Per cent	Material	Before incubation	After incubation		
	<i>per cent</i>			<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>per cent</i>	<i>per cent</i>
5-A	30	10	Saline	146	125	14.4	
	30	10	Adrenalin*	142	120	15.4	+7
10-A	30	10	Saline	100	85	15.0	
	30	10	Boiled extract	101	86	14.8	-1
3-A	35	10	Saline	168	144	14.3	
	35	10	Adrenalin*	167	140	16.2	+13
6-A	35	10	Saline	151	139	8.0	
	35	10	Adrenalin*	137	129	5.9	-25
8-A	35	10	Saline	108	93	13.9	
	35	10	Adrenalin*	107	89	16.8	+18
11-A	50	5	Saline	95	76	20.0	
	50	5	Adrenalin*	94	75	20.2	+1
	50	5	Boiled extract	94	75	20.2	+1
14-A	87	9	Saline	105	47	55.2	
	87	9	Adrenalin*	105	50	52.3	-5
	87	9	Boiled extract	105	54	48.5	-12

* Per cent of a solution containing 1 part of adrenalin in 2 million.

Tubes shaken 70 times per minute at 38°C., for 3 hours.

necessary to reduce the decoloration time of methylene blue by frog muscle *in vacuo* was 10^{-9} to 10^{-15} ; at 10^{-5} insulin showed an inhibitory effect.

An interesting relationship was found to occur in the blood-glucose-extract system. For a given concentration of blood there is an optimal concentration of extract necessary to give the maximal rate of glycolysis. Extract concentrations lower than the optimal do not give maximal decreases in glucose, while extract concentrations higher than the optimal inhibit the glycolytic reaction (table 1). A change in the concentration of blood shifted furthermore the optimal extract concentration. In general

it was observed that the greater the percentage of blood, the smaller the percentage of extract necessary to give maximal glycolysis. The reverse condition also held true: the smaller concentrations of blood required larger amounts of extract to give maximal effects.

In several experiments the lactic acid concentrations were determined before and after incubation. There was no significant change in the levels, however, nor any differences between the control and extract-containing tubes, and this line of investigation was not further pursued.

Cortical extract which had been boiled for two minutes in an open flask was found to be ineffective in increasing the control rates of glycolysis. Adrenalin solution (containing adrenalin in similar concentration—1:2,000,000—to that found in our extracts), used in control tubes in amounts equal to the quantities of extract used in the principal experiments, was also without effect on the (control) rate and amount of glucose decrease (table 2).

In one experiment in which the extract was added to blood-glucose in the presence of 0.002 M sodium cyanide the rate of glycolysis was found to be approximately the same as that occurring in the same system without the cyanide.

Statistical analysis of the glycolytic rates observed in extract-containing and control tubes indicates the validity of the results:

Mean, control tubes (M_c): 14.1 ± 0.98 per cent glycolysis.

Mean, extract tubes (M_e): 20.6 ± 1.21 per cent glycolysis.

Difference, $M_e - M_c$: 6.5.

Probable error of this difference: ± 1.55 .

SUMMARY

Cortico-adrenal extract increases the rate of glycolysis in the presence of normal defibrinated blood *in vitro* from 25 to 125 per cent (average 50 per cent) above that found in the control tubes.

The increased glucose utilization is a function of the relationship between the concentrations of blood and cortical extract: small amounts of blood require relatively large amounts of extract, and *vice versa*, in order to attain the maximal rate of glycolysis. Amounts of the extract greater than the optimal inhibit glycolysis.

Extracts which have been boiled in an open flask do not increase the rate of glucose disappearance.

Adrenalin used in dilution equal to that found in cortical extracts (1:2,000,000) is also ineffective.

Our cortico-adrenal extract changes from a pH of about 7.0 when freshly made to pH 4.5–5.0 when several weeks old. This is probably due to acid decomposition products of the small amount of adrenalin contained in the material.

BIBLIOGRAPHY

- BRITTON, S. W. AND H. SILVETTE. 1931. *This Journal*, xcix, 15.
1932. *Ibid.*, c, 693, 701.
- CORI, C. F. 1931. *Physiol. Rev.*, xi, 143.
- FOLIN, O. AND H. MALMROS. 1929. *Journ. Biol. Chem.*, lxxxi, 115.
- FRIEDEMANN, T. E., M. COTONIO AND P. A. SHAFFER. 1927. *Journ. Biol. Chem.*, lxxiii, 335.
- SWINGLE W. W. AND J. J. PEIFFNER. 1931. *This Journal*, xcvi, 164.

BLOOD-CELLULAR CHANGES IN ADRENAL INSUFFICIENCY AND THE EFFECTS OF CORTICO-ADRENAL EXTRACT¹

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The muscular incapacity, the circulatory disturbance and the disordered carbohydrate metabolism observed in adrenal insufficiency in animals and in man have been referred to in earlier reports (Britton, 1930; Britton and Silvette, 1931, 1932). The profound deficiencies which occur in the metabolism of carbohydrates have in our experiments appeared to be the dominant feature of adrenalectomy; the alleviatory effects of extract of the adrenal cortex appear furthermore to be directed mainly toward restoration of carbohydrate balance. That there are other important cortico-adrenal functions is none-the-less admitted. In a later report (Eagle, Britton and Kline, 1932) the influence of cortico-adrenal extract on muscular activity has been considered. Evidence on the extensive blood-cell changes in adrenal insufficiency, and the notable effects of extract administration, is now briefly presented.

The marked increase in blood concentration which follows adrenalectomy and the recovery to normal which is effected by cortico-adrenal extract have previously been reported (Britton and Silvette, 1931). These and other observations suggested the desirability of a more intimate study of probable variations in the blood-cellular elements under similar experimental conditions. Determinations have been made of the erythrocyte and leucocyte counts in cats under *a*, normal conditions; *b*, after adrenalectomy, and *c*, after extract treatment. Total and differential leucocyte determinations were carried out. Studies were further made on a number of rats and rabbits, and various control experiments were also performed. About fifty animals were studied. The adrenals were removed by a one-stage operation, the average survival period for cats being 6½ days. Cortico-adrenal extract made by a modified Swingle-Pfiffner procedure (Britton and Silvette, 1931) was used.

Erythrocytes. Red blood cell determinations were made in ten experiments on cats. The normal pre-operative counts for this group averaged 9,800,000 red cells per cu. mm. With the development of adrenal insuffi-

¹ Grateful acknowledgment is made of aid received in this investigation from the Grants-in-Aid Committee of the National Research Council.

ciency the number always rose considerably. The average count in the case of animals showing more or less severe symptoms was 15,500,000—an elevation of about 60 per cent above normal. This increase is in keeping with the reported observations on the changes in blood cell volume which follow removal of the adrenal glands (Britton and Silvette, 1931).

Cortico-adrenal extract was observed to bring about a notable reduction in the erythrocyte count in the course of a few hours, in some cases to approximately the pre-operative value. Adrenalin used in the concentration usually present in our cortical extract (1:2,000,000), and given in a large amount of fluid, failed to influence the cell values, however, to any significant degree. The protocol given below is illustrative. It will be noted that an increase in the red blood cell count began shortly after operation; while just before death, which supervened after extract had been stopped, the erythrocytes had almost doubled in number.

Cat 99; weight 2.2 kilos.

June 14, 9:00 a.m., normal r.b.c. 8.6 million; animal adrenalectomized
 June 15, 9:00 a.m., r.b.c. 8.6 million
 June 16, 9:00 a.m., r.b.c. 12.1 million, cat weak
 12:30 p.m., r.b.c. 14.6 million, very weak
 12:55 p.m., given 25 cc. adrenalin (1:2,000,000 intraperitoneally)
 3:00 p.m., r.b.c. 15.7 million, animal very weak, unable to stand
 3:15 p.m., 30 cc. cortico-adrenal extract (intraperitoneally)
 5:00 p.m., r.b.c. 14.3 million
 10:00 p.m., r.b.c., 9.8 million, marked improvement, cat walking about
 June 17, 9:00 a.m., r.b.c. 12.3 million; no injections
 June 18, 9:00 a.m., r.b.c. 14.5 million; no further extract given
 June 19, 9:00 a.m., r.b.c. 17.0 million. Cat died on this date

The data given in table 1 further show the changes produced by cortico-adrenal extract, in comparison with the negative results of adrenalin and saline injections. Glucose solution was also ineffective. The abnormally high erythrocyte levels which follow adrenalectomy are strikingly reduced by extract administration during the time that general recovery of the animal is brought about. It would seem possible and likely that the reductions occur because of blood dilution, although no fluid was given to the animals other than that contained in the extract.

Leucocytes: Total counts. Noteworthy changes were observed in the leucocyte counts in cats following adrenal removal and the subsequent development of symptoms of insufficiency. Normal total and differential determinations were made before operation, and continued daily after excision of the adrenal glands.

In a few cases the total white cell counts appeared abnormally high, varying between 14,000 and 28,000 per cu. mm., before operation. The normal range in most cases appeared to be between 7,000 and 14,000 cells.

In eight experiments on animals which were carefully followed, the leucocyte count averaged 12,400 before operation. Removal of the adrenals resulted in a reduction in these cases within a few days to an average of

TABLE 1

Showing the marked increase in erythrocytic counts (given in millions per cu. mm.) after adrenalectomy, and the influence of various injected materials

CAT NUMBER	NORMAL ERYTHROCYTES	ERYTHROCYTES IN ADRENAL INSUFFICIENCY	MATERIAL INJECTED	ERYTHROCYTES AFTER TREATMENT	DIFFERENCE AFTER TREATMENT	PERCENT-AGE DIFFERENCE
95	11.2	18.6	Adrenal extract	13.9	-4.7	-25
97	6.9	17.6	Adrenal extract	13.3	-4.3	-24
99	8.6	15.7	Adrenal extract	9.8	-5.9	-38
92	11.9	14.1	Adrenalin (1:2,000,000)	14.2	+0.1	+1
97	6.9	17.3	Adrenalin (1:2,000,000)	17.1	-0.2	-1
99	8.6	14.6	Adrenalin (1:2,000,000)	15.7	+1.1	+7
93	11.3	14.0	Saline (0.9 per cent)	13.3	-0.7	-5
96	9.4	13.8	Saline (0.9 per cent)	13.6	-0.2	-1
100	8.9	14.7	Saline (0.9 per cent)	17.3	+2.6	+18

Cortico-adrenal extract and control solutions were given by intraperitoneal injection in dosage of 10 cc. per kilo.

TABLE 2

Changes in total leucocytic counts after adrenalectomy and the influence of cortico-adrenal extract and of adrenalin (controls)

CAT NUMBER	TOTAL LEUCOCYTES (NORMAL)	LEUCOCYTES BEFORE INJECTION (AFTER OPERATION)	MATERIAL INJECTED	LEUCOCYTES AFTER INJECTION	DIFFERENCE AFTER INJECTION	AVERAGE DIFFERENCE
71	10,400	7,600	Adrenal extract	11,200	+3,600	+6,500
71		10,800	Adrenal extract	16,800	+6,000	
71		11,200	Adrenal extract	24,200	+13,000	
76		8,800	Adrenal extract	12,400	+3,600	
76		12,400	Adrenal extract	18,600	+6,200	
56	20,800	10,400	Adrenalin	5,600	-4,800	-2,900
58	7,800	5,800	Adrenalin	4,000	-1,800	
63	10,800	9,200	Adrenalin	6,800	-2,400	
64	10,000	5,600	Adrenalin	2,800	-2,800	

7,000 cells. In one animal there was a fall in the total count to 2,800 and in another to 4,000 cells before death.

The effects of administering cortico-adrenal extract to animals which were showing symptoms of adrenal insufficiency were invariably striking. Associated with the process of recovery, the total white counts were con-

siderably augmented—in some cases to relatively high limits. Cat 71 showed an increase, for example, from 7,600 to 24,200 cells after three injections of extract (table 2). In control experiments in which adrenalin was injected no such responses were observed; the leucocyte counts continued to fall, indeed, until the animals succumbed.

Differential leucocyte counts. Adrenalectomy resulted in very significant changes in the differential leucocyte counts. Within a day or two after operation, the neutrophils were found to be reduced, and the lymphocytes correlatively increased in percentage. These changes were progressive until wide variations from the normal levels were observed with the development of symptoms of insufficiency. In some cases the neutrophils

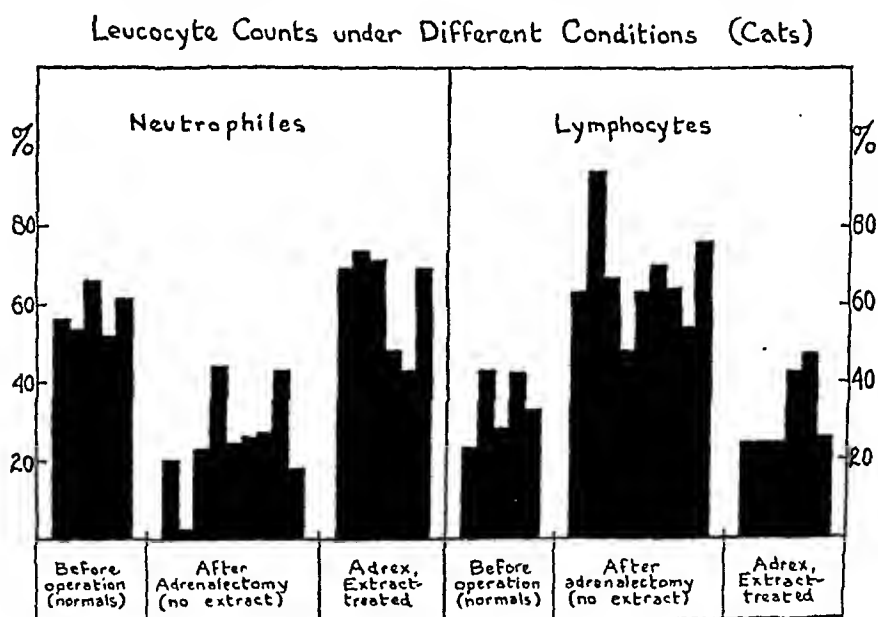


Fig. 1

almost entirely disappeared; they were reduced to the vanishing level of 3 per cent, with the appearance of adrenal insufficiency, in the case of animal 46.

The administration of cortico-adrenal extract brought about a complete reversal of the disorganized blood-cellular picture observed in adrenal insufficiency. Several hours were often necessary to effect the complete change; it took place coincidentally, however, with the restoration of the animal to normal activity. The changes are shown in the experiment outlined in table 3, and in summary in table 4.

Shifts in the differential cell count toward the normal were sometimes brought about by adrenalin administration. These changes were very slight, however, and always short-lived.

TABLE 3

Experiment: Cat 46: Showing the effects on the differential leucocyte counts of adrenalectomy and of the administration of cortico-adrenal extract

DATE AND TIME	CONDITIONS	MYELO-CYTES	NEUTRO-PHILS	EOSINO-PHILS	BASOPHILS	LYMPHO-CYTES	MONONU-CLEARS
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Mar. 12	Normal	0	53	2	1	43	1
Mar. 12	Adrex.*						
Mar. 21		1	12	5	1	74	7
Mar. 22		0	3	0	0	94	3
Mar. 22	Extract given						
5 p.m.							
6 p.m.		0	16	3	1	74	6
11.30		1	24	1	0	63	11
Mar. 23	Extract given	0	23	1	0	67	9
9 a.m.							
11 a.m.		0	39	1	0	53	7
12 m.		0	60	2	1	29	8
9.30 p.m.		0	69	1	0	24	6

* Bilateral adrenalectomy.

TABLE 4

*Showing the fall in neutrophils and the increase in lymphocytes after adrenalectomy, and the restititional effects of administering cortico-adrenal extract
(Adrenalin was ineffective)*

CAT NUMBER	NORMAL		BEFORE EXTRACT (ADRENAL INSUFFICIENCY)		AFTER EXTRACT	
	Neutrophils	Lymphocytes	Neutrophils	Lymphocytes	Neutrophils	Lymphocytes
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
46	53	43	3	94	24	63
46			23	67	69	24
47	66	28	44	47	73	24
47			24	64	70	24
49	52	42	26	70	48	43
49			27	64	43	47
51	61	33	43	54	69	25
51			18	76	32	62
76	80	14	62	36	91	8
Average. ...	63	32	30	64	58	36

Positive effects of adrenal extract on the differential white counts were observed furthermore in normal and adrenalectomized white rats and in normal rabbits, as well as in operated cats. In adrenalectomized rats adrenalin appeared to be able to raise the (previously reduced) neutro-

phile counts, although not as markedly as cortico-adrenal extract. The difference in reaction to adrenalin between the rat and the cat may possibly be due to the wide difference in the normal neutrophilic content of the blood of these animals. The normal rat possesses approximately one-half the percentage of neutrophiles present in normal cat blood. Comparison of the differential effects of various substances on the blood of these forms may thus be impossible. Accessory cortico-adrenal tissue may also complicate the conditions in the case of (apparently) adrenalectomized rats.

Splenectomy. In several control cats the spleen was removed and the blood thereafter studied for possible changes which might be attributed to the absence of this organ. No significant variations from the normal leucocyte levels were observed, although subsequent adrenalectomy quickly brought about changes similar to those noted above. In these splenecto-adrenalectomized animals cortico-adrenal extract was effective in restoring the normal leucocyte levels, while control experiments with adrenalin were negative.

DISCUSSION. It is interesting to note that Addison in his classical monograph on adrenal disease (1855) indicated that the circulatory manifestations of the condition were of considerable importance. Anemic and other features of the disease led him to the belief, indeed, that the adrenal glands were "directly or indirectly concerned in sanguification." The present data give support to this very early prediction.

Extensive changes in the leucocytic content of the blood of adrenalectomized cats have been observed by Zwemer and Lyons (1928). Removal of the adrenals in their cases was followed, in the longer-surviving animals, by a decrease in the percentage of polymorphonuclear neutrophiles and an increase in the percentage of lymphocytes. The total number of leucocytes was found to be decreased, as a rule, in adrenal insufficiency. Stewart (1926) and Rogoff and Stewart (1926) have called attention to the marked decrease in the plasma content of the blood following adrenalectomy in the dog. These workers further noted considerable increases in the erythrocyte counts, and also in the hemoglobin percentage and specific gravity of the blood. Observations made on the cat in this laboratory (Britton and Silvette, 1931) are in agreement with these findings.

It seems likely that the changes in red blood cell concentration which occur in adrenal insufficiency are indications of the extensive shifts in water balance in the organism. The profound effects of the operation on the leucocytic levels probably represent blood-cellular shifts which are more specifically related, however, to the adrenalectomized animal. The results indicate the possibility, indeed, of a neutrophilogenic failure in the adrenalless organism, with an apparent related lymphocytosis. The possible involvement of the bone marrow and other tissues in adrenal insufficiency is thus indicated, and this phase of the problem is now being investigated.

It may be worth while to consider whether the condition of neutrophilopenia which supervenes in adrenalectomy has any relationship to the condition of "agranulocytosis" as observed in man. The individual's "chief cellular defense mechanism, the neutrophilic leucocyte" (Doan, 1932) is profoundly disturbed in both cases. The evidence now emphasizes the prompt relief afforded by treatment of the experimental animal with cortico-adrenal extract, although the specificity of the reaction is not at present considered. General factors, possibly those involving tissue permeability, may nevertheless be concerned.

It seems reasonable to suppose that such a highly differentiated (and probably relatively unstable) tissue as the adrenal cortex may be disorganized oftener and more readily than suspected, and such disorganization may indeed be implicated in neutropenic conditions observed in man. The pathological effects of adrenal removal are widespread and profound, and it would appear likely that many diseases of now unknown etiology may be explicable on the basis of cortico-adrenal hypofunction or dysfunction. The increasing availability and knowledge of extracts of the adrenal cortex surely suggest that more extensive tests of the therapeutic value of the material be now undertaken.

SUMMARY

Adrenalectomy produces marked numerical changes in the cellular elements of the blood. With the development of symptoms of adrenal insufficiency, the erythrocytes increase commonly from 50 to 100 per cent; this change is probably due to fluid loss from the blood. The total leucocyte counts are meanwhile found to be decreased to a similar extent. There are pronounced reductions in the neutrophile counts, sometimes almost to the disappearing point. The lymphocytes show a concomitant increase in percentage.

The administration of cortico-adrenal extract to animals suffering from severe adrenal insufficiency and showing the above profound blood-cellular disorganization resulted in complete restitution of the normal cell values. Recovery of the blood cell elements to normal values was coincident with general improvement in the condition of the animal.

Control experiments with adrenalin, glucose and saline solutions were negative, with the exception of the partly effective action of adrenalin on the white rat.

There were no noteworthy leucocytic changes in splenectomized controls. Splenecto-adrenalectomized animals showed responses after operation and after extract treatment, however, which were similar to those noted above.

The possibility that the neutrophilopenia of adrenal insufficiency is related to the clinical condition of "agranulocytosis" is considered.

BIBLIOGRAPHY

- ADDISON, T. 1855. On the constitutional and local effects of disease of the supra-renal capsules.
- BRITTON, S. W. 1930. *Physiol. Rev.*, x, 617.
- BRITTON, S. W. AND H. SILVETTE. 1931. *This Journal*, xcix, 15.
1932. *Ibid.*, c, 701.
- COREY, E. L. AND S. W. BRITTON. 1931. *This Journal*, xcix, 33.
- DOAN, C. A. 1932. *Journ. Amer. Med. Assoc.*, cxix, 194.
- EAGLE, E., S. W. BRITTON AND R. KLINE. 1932. *This Journal*, cii, 707.
- ROGOFF, J. M. AND G. N. STEWART. 1926. *This Journal*, lxxviii, 711.
- STEWART, G. N. 1926. *Journ. Pharm. Exper. Therap.*, Abel Memorial Vol.
- ZWEMER, R. L. AND C. LYONS. 1928. *This Journal*, lxxxvi, 545.

THE INFLUENCE OF CORTICO-ADRENAL EXTRACT ON ENERGY OUTPUT¹

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In earlier papers we have presented brief reports which indicate a significant influence of cortico-adrenal extract on the ability of dogs to perform muscular work (Britton and Silvette, 1931; Eagle and Britton, 1932). Continued improvement in methods of preparing the extract has now made available a material which is sterile, protein-free and contains practically no adrenalin, and which possesses significant effects on many bodily activities. We have thus been able recently to complete a series of observations begun about two years ago on the energy output of normal dogs before and after injection of adrenal extract of known potency, made according to a modified Swingle-Pfiffner method (Britton and Silvette, 1931).

The procedures for determining the working capacity of animals were similar to those used by Campos, Cannon, Lundin and Walker (1929), with whom one of us was earlier associated. These investigators noted the effects of adrenalin, glucose, insulin and various operations on the maximal running ability. In our experiments, dogs were first trained over a period of several weeks to run in a treadmill. The apparatus consisted of a cage enclosing an endless belt which was moved by means of an electric motor; through a rheostat the travelling speed of the belt could be varied from 110 to 224 meters per minute. The number of revolutions of the measured belt, inclined at an angle of 10 degrees, was calculated from the recorded revolutions of one of the pulleys: 1000 revolutions of the belt corresponded to a counter reading of 3948.

The animals, which had been kept fasting before an experiment for a period of 18 to 20 hours, were first allowed to become quiet before pulse readings and blood samples were taken. Blood was taken from the peripheral ear vessels, and the sugar was determined according to the method

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of Folin and Malmros (1929). After the "starting" pulse readings and blood samples were obtained, the dogs were allowed to run in the treadmill until, with four stops being made for observation as hereafter noted, they eventually became exhausted. The end-point used was that described by Campos, Cannon *et al.*: it was indicated by the animal lying down and refusing to run further, by refusing to rise after the treadmill had been stopped, or by promptly lying down and refusing to run when the mill had been restarted. The performance of the dogs and their condition after each bout of exercise were also designated for purposes of comparison according to the method employed by the foregoing workers as follows:

Performance: V—vigorous running; S—steady running; I—irregular running; R—refusal to run.

Condition at end of run: A—active; W—weary, panting; T—tired, panting heavily; F—fatigued; E—exhausted.

In some cases it was not possible to denote the condition of the animal adequately by one letter, and two were therefore used.

Unlike the procedure employed by the Harvard investigators, we stopped the treadmill at indicated times during the experiment so that checks on blood sugar and pulse could be taken. Thus, an animal was permitted to run as long as its running behavior could be designated by "V" (vigorous); when vigorous running was marred by more than rare stops, the treadmill was stopped and readings and samples taken. After five minutes' rest, the machine was again started and the dog permitted to run until its behavior was no longer denoted by "S." The same procedure was followed for the conditions signified by the letters "I" and "R." The dog therefore ran for four periods, and received three rest periods of five minutes each. As will be shown later, under these conditions the animal ran apparently with willingness to the point of exhaustion; further, uniformity was maintained throughout the series. Comparison of the performances of an animal throughout its experimental course was readily possible.

The animals were allowed to run in the mill once every 3 to 5 days until, after the process of training had extended over several weeks, their working ability was maintained at a fairly uniform level. After this standard working capacity, representing a maximum energy output which remained constant for several successive experiments, had been definitely established, cortico-adrenal extract was administered intraperitoneally (with one exception), and determinations of running ability again made.

It was thought that careful study of a few selected animals over fairly long periods would be of greater value than the use of many over a relatively short time. Three dogs have thus been studied over periods of four, five and seven months respectively, with preliminary training periods

of several weeks in each case. Small dogs of about 7 kilos in weight were used in order to conserve the (expensive) extract, and also to avoid the necessity of attending to very long runs (of 4-8 hours), which we found could be readily carried out by larger animals. Some dogs which we tested appeared to be almost tireless runners.

In each experiment, calculation of the total expenditure of energy in excess of basal metabolism was carried out. This was possible through the use of average factors obtained from the determinations made by various investigators. Zuntz (1903) has reported that for moving 1 kilogram of a dog weighing 29.35 kgm. through a horizontal distance of 1 meter the energy expenditure in excess of basal metabolism did not vary when the running rate was 57.4 meters per minute or 101.1 meters per minute. Our rates of speed did not show nearly such a wide variation, and it can be considered that the slight changes which did occur exerted little or no influence on the results.

The average of the determinations made by Zuntz (1903) and Slowtsoff (1903) was 0.67 kgm-m.; Frentzel and Reich (1900) reported a value of 0.501; and Anderson and Lusk (1917) 0.58, with a maximum variation of ± 1.7 per cent. These figures have been derived from experiments on animals of various weights running at widely different rates. Campos, Cannon *et al.*, using dogs from 10 to 12 kgm. in weight, considered 0.6 kgm-m. as the energy expenditure to move 1 kgm. of dog 1 meter of horizontal distance. Although our dogs weighed somewhat less, it was considered that the same value (0.6 kgm-m.) could be justifiably used. Since we are interested primarily in the percentage change of total energy output after administration of the extract, the use of a somewhat higher factor would involve no difference in the final analysis.

Considering the values for vertical work, Zuntz and Slowtsoff have noted that the energy output for elevations from 14.2 to 23.6 per cent is practically constant. The average was approximately 3 kgm-m. per kgm. of dog raised 1 meter. From the foregoing data it has thus been possible to calculate the expenditure of energy per kilogram body weight in excess of basal metabolism as follows:

$$E = (0.6 \times a \times \cos 10^\circ) + (3 \times a \times \sin 10^\circ) = a \times 1.11$$

where a equals the distance run at an elevation of 10 degrees of the treadmill platform. Although, as has been pointed out by the Harvard workers, "this value is probably minimal and approximately represents the work of transporting the body, but does not include that involved in the functioning of the respiratory muscles," it amply serves the purpose of our experiments in which a percentage change in energy output is the condition to be noted.

Our earlier observations carried out on dog "Brownie" are given in brief

in table 1. After preliminary training experiments extending over about four weeks a standard working capacity had been attained. Thus, an energy output of approximately 12,000 kgm-m. represented the normal for this small 7-kilo animal. After administration of cortico-adrenal extract,

TABLE 1

Data of experiments on dog "Brownie" (male, 7 kilos), showing normal running capacity and effects of injection of cortico-adrenal extract
(See text for injection times)

DATE AND INJECTION	RUNNING TIME	RUNNING RATE	ENERGY* IN 1000 KGM-M.	BLOOD SUGAR	
				Start of run	End of run
	minutes	m. per minute		mgm. per 100 cc.	mgm. per 100 cc.
1.23.31	101	110	12.3	—	—
1.30.31	94	116	12.1	—	—
{ 2. 5.31	—	—	—	—	—
{ (10 cc. Ext.)†					
{ 2.11.31	97	119	12.8	84	96
{ (20 cc. Ext.)					
2.21.31	171	121	23.0	131	74
2.25.31	137	121	18.4	94	64
2.28.31	102	118	13.3	94	63
{ 3. 2.31	103	121	13.8	91	89
{ (20 cc. Ext.)					
3. 5.31	110	122	14.8	88	83
3. 7.31	138	125	19.2	92	74
3.12.31	190	124	26.2	88	74
3.15.31	147	127	20.7	85	83
3.18.31	139	129	19.9	75	64
3.22.31	132	129	18.9	99	96
4. 4.31	116	125	16.1	91	73
4. 8.31	120	122	16.2	92	85
4.15.31	117	122	15.8	95	80
4.23.31	97	125	13.5	84	75
{ 4.30.31	91	124	12.5	83	74
{ (20 cc. Ext.)					
5. 5.31	155	122	21.0	80	73
5. 9.31	96	121	12.9	89	85
5.12.31	97	120	13.0	88	79
5.20.31	95	119	12.5	87	76

* Energy calculated in 1000 kilogram-meters in excess of basal metabolism (see text).

† Intravenous injection; all others intraperitoneal.

the working capacity increased to a figure almost twice as high, to fall again within two weeks or so to the basal working level. Injections were given on the date immediately above the amount noted in each case, and just before placing the animal in the treadmill except in the experiments

on "Snookie." Two further tests showed similar results—considerable increments in energy output after extract treatment, with subsequent diminutions to the normal level. The running time, it may be observed, was increased about 80 per cent, and the distance run to approximately the

TABLE 2

Experiments carried out on dogs "Lady" and "Snookie," showing effects of treatment with cortico-adrenal extract

	DATE AND INJECTION	RUNNING TIME	RUNNING RATE	ENERGY IN 1000 KGM-M.	BLOOD SUGAR	
					Start of run	End of run
		minutes	m. per minute		mgm. per 100 cc.	mgm. per 100 cc.
Dog, "Lady".....	4.14.32	52	120	6.9	99	102
	4.18.32	72	98	7.9	111	105
	4.21.32	71	105	8.3	109	98
	(25 cc. Ext.)					
	4.25.32	142	102	16.1	114	101
	4.28.32	112	96	11.9	96	89
	5. 2.32	99	105	10.5	104	98
	5.31.32	72	99	7.9	83	87
	6. 4.32	77	95	8.1	90	84
	5.17.32	89	82	8.1	74	69
	5.24.32	101	85	9.5	79	74
	5.27.32	92	77	7.9	84	85
	(20 cc. Ext.)					
	5.31.32	181	80	16.1	83	65
Dog, "Snookie"....	6. 4.32*					
	6. 7.32	95	79	8.3	89	72
	6.17.32	96	77	8.2	92	78
	6.21.32	97	87	9.3	88	83
	(35 cc. Ext.)					
	6.24.32	183	89	18.1	112	86
	6.28.32	137	77	11.7	92	88
	7. 1.32	110	78	9.5	89	82
	7. 5.32	98	82	8.9	84	83
	7. 8.32	92	80	8.2	84	80
	7.12.32	90	78	7.8	86	80

* Dog developed fits of sneezing and run was stopped.

same extent. The rate of running was kept within a small range, thus practically eliminating the possibility of error in determinations.

The blood sugar almost invariably showed a fall at the end of exercise, in comparison with the initial (fasting) level.

Experiments which we carried out a year later than the foregoing on two other dogs and with improved adrenal extracts resulted similarly. By

reference to tables 2 and 3 it may be observed that the working capacity was doubled after extract administration. In the experiments on "Snookie" the injections were given at 9 a.m., and the dog was run at 3 p.m. on the same day. Increments in energy output in these cases appeared from 6 to 9 hours after extract was given, it will be noted. An animal exercised immediately after an injection, however, did not usually show a notable increase in running capacity on the same day.

The percentage increases in energy output after extract injection over the average of the two pre-extract runs were as follows: "Brownie"—92 per cent, 94 per cent and 62 per cent; "Lady"—100 per cent; "Snookie"—85 per cent and 108 per cent. The average increase in the experimental series was 90 per cent.

TABLE 3

Data on running time and distance run in different experiments before and after extract injection

DOG	RUNNING TIME			DISTANCE RUN		
	Before extract	After extract	Increase	Before extract	After extract	Increase
	minutes	minutes	per cent	kgm.-m.	kgm.-m.	per cent
"Brownie".....	94	171	82	10.6	20.3	92
	103	190	85	12.5	23.6	93
	91	155	70	11.2	18.9	69
"Lady".....	71	142	100	7.4	14.5	96
"Snookie".....	92	181	97	7.1	14.5	104
	97	183	89	8.4	16.3	94

There appears little doubt that the adrenal glands are intimately and possibly specifically related to neuromuscular activities. In disease of the organs in man (*morbis Addisonii*), chronic and progressive asthenia is said to be the outstanding clinical characteristic observed. It has been shown, however, that remarkable recoveries may be brought about by administration of extract of the adrenal cortex (Rowntree *et al.*, 1931). Animals from which the adrenals have been removed are able to carry out only a small fraction of the work performed by normal individuals (Britton, 1930). Decline in the muscular ability of adrenalectomized animals sets in, according to our experience, very shortly after the operation, although survival may continue for ten days or more. Even the severe prostration of adrenal insufficiency may nevertheless be overcome by large doses of cortico-adrenal extract (Swingle and Pffiffer, 1931; Britton and Silvette, 1931). Metabolic studies are in keeping with these observations, and again are indicative of an adreno-neuromuscular relationship.

SUMMARY

The influence of cortico-adrenal extract on the energy output of dogs running in a treadmill has been studied. Three animals were observed for periods of four, five and seven months. After the standard running capacity had been established, the effects of adrenal extract were tested. Intraperitoneal injection of the extract was found to augment markedly the energy output up to 100 per cent or more above the normal. The average increase in six series of experiments was 90 per cent.

The running time and the distance run were also increased about 90 per cent. In one instance an animal which regularly ran a distance of about 4 miles before treatment covered over 9 miles under the influence of cortico-adrenal extract. The effects were noticeable for ten to fifteen days after injection.

Blood sugar almost invariably fell during prolonged running and its course was not notably altered by cortico-adrenal extract.

BIBLIOGRAPHY

- ANDERSON, R. J. AND G. LUSK. 1917. *Journ. Biol. Chem.*, xxxii, 421.
BRITTON, S. W. 1930. *Physiol. Rev.*, x, 617.
BRITTON, S. W. AND H. SILVETTE. 1931. *This Journal*, xcix, 15. *Ibid.*, xcvii, 507.
1932. *Ibid.*, c, 693, 701.
CAMPOS, F. A. deM., W. B. CANNON, H. LUNDIN AND T. T. WALKER. 1929. *This Journal*, lxxxvii, 680.
EAGLE, E. AND S. W. BRITTON. 1932. *Science*, lxxv, 221.
FOLIN, O. AND H. MALMROS. 1929. *Journ. Biol. Chem.*, lxxxiii, 115.
FRENTZEL, J. AND F. REICH. 1900. *Pflüger's Arch.*, lxxxiii, 494.
ROWNTREE, L. G., C. H. GREENE, W. W. SWINGLE AND J. J. PFIFFNER. 1931. *Journ. Amer. Med. Assoc.*, xcvi, 231.
SLOWTZOFF, B. 1903. *Pflüger's Arch.*, xcv, 158.
SWINGLE, W. W. AND J. J. PFIFFNER. 1931. *This Journal*, xcvi, 153.
ZUNTZ, N. 1903. *Pflüger's Arch.*, xcv, 192.

STUDY ON THE METABOLISM OF GLUTATHIONE

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It has long been known that protein-free tissue extracts give a purple color with sodium nitroprusside and ammonia. This reaction, being characteristic for sulfhydryl groups, was considered by Arnold (1910) as due to free cysteine. Though the reduction of cystine to cysteine seems to be the first step in the catabolism of this sulfur containing constituent of proteins, there has been no experimental evidence for the general occurrence of cysteine in tissues. Using the delicate naphthoquinone sulfonic acid test devised by Sullivan (1926), Thompson and Voegtlin (1926) came to the conclusion "that tissues do not contain an appreciable amount of either cystine or cysteine." This statement must be accepted with reservation, since Hunter and Eagles (1927) isolated pure cystine from pig's liver, taking great care to exclude secondary hydrolysis of protein. Hopkins (1921) showed that the substance responsible for the nitroprusside reaction is of more complex nature. He succeeded in isolating from yeast, muscle and liver a crystalline compound, to which he gave the name glutathione. According to newer investigations (Hopkins, 1929; Kendall, MaKenzie and Mason, 1929) it is a tripeptide of glycine, glutamic acid and cysteine. The physiological importance of this substance lies in the rôle which it plays in oxidation-reduction processes of cells. Its metabolic function is dealt with in a recent paper by Hele and Pirie (1931). The relatively high concentration of glutathione in liver, testes, adrenals and lens of the eyes, and the general distribution of glutathione in tissues, led these investigators to assume that it is a metabolic end product. They studied the oxidation of active and racemic cystine, cysteine and some derivatives as well as reduced and oxidized glutathione in the animal body. The rate of oxidation of these sulfur compounds varied with the animal and the mode of administration. An average of about 70 per cent of the sulfur of cystine appeared as sulfates in the urine; for cysteine the figures are slightly lower. Glutathione, in the reduced and oxidized form, was the most completely oxidized of all the compounds studied. An average of 72 per cent of its sulfur was recovered as sulfates. Great differences in the excretion of neutral sulfur were observed. However, no attempt to study its nature was made.

The aim of the present study was to investigate the effect of a high protein diet, and of orally and intravenously administered glutathione and its constituent amino acids on the level of blood glutathione, and to investigate the rate of oxidation of these substances in the animal body.

EXPERIMENTS. Adult bitches were used throughout this investigation. The blood glutathione was determined by the method of Mason (1930). For a convenient application see Schelling (1932). The total and inorganic sulfates in urine were determined, after the removal of phosphates, by Fiske's (1921) method. A part of the glutathione used was prepared according to the method of Pirie (1930). The remainder was a commercial brand of the Eastman Kodak Company. Judged from melting point determinations these substances were pure. The cysteine hydrochloride was obtained from the Pfanstiehl Chemical Company. Glutamic acid as well as glycine were prepared according to the methods described in *Organic Syntheses*, vol. v, page 63, and vol. iv, page 31, respectively.

TABLE 1
Effect of a high protein meal on blood glutathione of dogs

	BLOOD GLUTATHIONE			
	Dog 32, 24.5 kilos	Dog 33, 15.8 kilos	Dog 52, 20.4 kilos	Dog 55, 17.6 kilos
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
Fasting.....	14.2	26.8	31.2	21.0
1st hour.....	13.9	24.2	33.3	22.7
2nd hour.....	14.1	26.1	33.8	26.9
4th hour.....	25.8	29.6	31.2	25.2
6th hour.....	21.4	20.7	29.9	17.9

A. Blood glutathione following the ingestion of a high protein meal. The dogs were kept for a preliminary period of 7 days on a diet of milk and soda crackers in amounts providing 80 calories per kilo body weight. This food was then withheld for one day and the following day 500 grams ground lean beef were given in one portion. Blood samples for the determination of glutathione were taken from the fasting animal and at intervals of 1, 2, 4 and 6 hours after the meal. The findings are shown in table 1. With one exception (dog 32) the increase in blood glutathione is slight and reaches a peak somewhere between the second and fourth hour after the ingestion of the meat.

B. Effect of orally and intravenously administered glutathione and its constituent amino acids on the blood glutathione and the excretion of total nitrogen and total and inorganic sulfates in the urine. The dogs were kept on a standard diet of milk and soda crackers; in experiment 3 a ground and dried mixture of equal parts of fresh, trimmed beef hearts and soda cracker

TABLE 2

Changes in blood glutathione, inorganic and total sulfates in urine of dogs after oral and intravenous administration of glutathione and its three constituent amino acids

DATE	TOTAL NITROGEN	INORGANIC SULFATES	TOTAL SULFATES	BLOOD GLUTATHIONE					COMMENTS
				Fast- ing	1st hour	2nd hour	4th hour	6th hour	

Experiment 1. Dog, 26.2 kilos. Food: 1 quart of milk, 348 grams of soda crackers

1932	grams	gram	gram	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	
Jan. 28	7.68	0.362	0.373						
Jan. 30	7.63	0.368	0.381						
Jan. 31	7.75	0.361	0.374						
Feb. 1	7.93	0.448	0.466						1 gram glutathione by mouth (0.136 gram N, 0.104 gram S) 1 gram glutathione by mouth
Feb. 2	7.61	0.372	0.384						
Feb. 4	7.77	0.432							
Feb. 5	7.59	0.389	0.397						
Feb. 7	7.57	0.372							
Feb. 8	7.61	0.364							
Feb. 9	7.71	0.399							0.4 gram glutathione intravenously (0.054 gram N, 0.042 gram S) 1.57 grams cysteine-HCl, 1.47 gram glutamic acid, 0.75 gram glycine p.os. (0.42 gram N, 0.32 gram S)
Feb. 12	7.62	0.366	0.378						
Feb. 14	7.60	0.364							
Feb. 15	8.01	0.651							
Feb. 16	7.62	0.366							

Experiment 2. Dog, 21.8 kilos. Food: 1 quart of milk, 213 grams of soda crackers

Feb. 21	6.00	0.211							
Feb. 22	6.04	0.206	0.219						
Feb. 23	6.30	0.336	0.350	29.3	33.6	33.6	33.1	33.1	1 gram glutathione by mouth (0.136 gram N, 0.104 gram S)
Feb. 24	6.10	0.276	0.288						
Feb. 25	6.00	0.268	0.280						
Feb. 26	6.19	0.294	0.304	31.2	32.2	33.6	33.3	32.8	0.5 gram glutathione intravenously (0.068 gram N, 0.052 gram S)
Feb. 27	6.08	0.271	0.291						
Feb. 28	6.14	0.274							
Feb. 29	5.91	0.275	0.285						
March 1	6.10	0.387		36.5	38.7	38.2	37.9	37.1	1 gram cysteine-HCl by mouth (0.113 gram N, 0.205 gram S)
March 2	6.08	0.312							
March 3	6.10	0.269	0.279						
March 4	6.03	0.271	0.282						
March 5	6.53	0.534	0.541	34.2	33.6	33.2	33.6	34.0	1.57 grams cysteine-HCl, 1.47 gram glutamic acid, 0.75 gram glycine p.os. (0.42 gram N, 0.32 gram S)
March 6	6.32	0.292	0.299						
March 7	6.09	0.280	0.298						
March 8	6.12	0.275	0.291						

TABLE 2—Concluded

TABLE 1—Continued

DATE	TOTAL NITROGEN	INORGANIC SULFATES	TOTAL SULFATES	BLOOD GLUTATHIONE					COMMENTS	
				Fast- ing	1st hour	2nd hour	4th hour	6th hour		
Experiment 3. Dog, 20.4 kilos. Food: 400 grams beef heart-cracker mixture										
1932	grams	gram	gram	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.		
March 10	9.76	0.396	0.402							
March 11	9.73	0.407	0.425							
March 12	9.67	0.389	0.405							
March 14	9.80	0.484	0.499							1 gram glutathione by mouth (0.136 gram N, 0.104 gram S)
March 15	9.71	0.410	0.425							
March 16	9.76	0.401	0.416							
March 17	9.66	0.450	0.459	27.3	28.7	29.4	29.0	28.5		0.5 gram glutathione in- travenously (0.068 gram N, 0.052 gram S)
March 18	9.58	0.404	0.417							
March 19	9.62	0.405	0.416							
March 20	9.59	0.405	0.418							
March 21	10.34	0.697	0.708	24.5	28.7	29.4	29.6	27.8		1.57 gram cysteine-HCl by mouth (0.14 gram N, 0.32 gram S)
March 22	9.61	0.430	0.441							
March 23	10.87	0.676	0.694	29.2	30.7	31.0	30.9	31.2		1.57 gram cysteine-HCl, 1.47 gram glutamic acid, 0.75 gram glycine p.os (0.42 gram N, 0.32 gram S)
March 24	9.82	0.418	0.431							

meal was used. When sulfur and nitrogen excretion became constant the administration of the substance under investigation was started. For the oral administration the glutathione and the amino acid mixture were added to a separate small portion of the standard diet. This mixture was usually consumed in a few minutes; the remaining bulk of the diet was then fed. For the intravenous administration the substances were dissolved in the necessary amount of distilled water and adjusted to pH approximately 6.0 by tenth normal sodium hydroxide. The 24 hour urine periods were always terminated by catheterizing and washing the bladder. The results obtained in three dogs are summarized in table 2. From this it is seen that orally and intravenously administered glutathione, as well as a mixture of its three constituent amino acids, only slightly increased the blood glutathione. The increase in sulfate excretion over the average of the control days indicates that from 67 to 86 per cent of the sulfur of glutathione given per os is excreted as inorganic sulfate within a 48 hour period. Figures of about the same magnitude were obtained on intravenous administration; a top figure of 92 per cent was reached in experiment 3.

DISCUSSION. The slight rise in blood glutathione following administration of cysteine, glycine and glutamic acid may, of course, be due to un-

changed cysteine which gives the same color reaction as glutathione. It has been repeatedly shown by different investigators that the sulfur of orally administered cystine and cysteine undergoes oxidation in the animal body and is excreted by the kidneys primarily as inorganic sulfate. The degree of oxidation and excretion varies somewhat with the species of animal and the composition of the food, especially in regard to the nitrogen-sulfur ratio.

It was *a priori* to be expected that glutathione given per os would undergo the usual catabolic changes of peptides in the gastro-intestinal tract, i.e., splitting into amino acids. The relatively slight increase in blood glutathione after oral administration of glutathione suggests that only a small part of the compound passes through the intestinal wall unchanged, or that any portion absorbed is stored in the liver which, according to Thompson and Voegtlin (1926), is one of the organs richest in glutathione. On the other hand the general occurrence of glutathione in tissues and blood leads to the assumption that it is synthesized where a physiological need arises. The factors governing this synthesis in relation to requirements are not yet known, and further investigation in this direction will be awaited with interest.

In conclusion the results may be summarized as follows:

1. A high protein meal does not materially increase the blood glutathione in dogs during the digestion period.

2. Reduced glutathione, orally or intravenously administered in amounts of 0.5 to 1 gram, increases the blood level of glutathione only 2 to 4 mgm. per 100 cc. The sulfur of glutathione is oxidized to the extent of 70 to 90 per cent and appears in the urine as inorganic sulfate within the 48 hour period. The fate of the sulphur of glutathione is, similarly to that of cysteine, administered with equimolecular parts of glycine and glutamic acid under the same dietary conditions.

BIBLIOGRAPHY

- ARNOLD, V. 1910-1911. *Zeitschr. f. physiol. Chem.*, lxx, 314.
FISKE, C. H. 1921. *Journ. Biol. Chem.*, xlvii, 59.
HELE, T. S. AND N. W. PIRIE. 1931. *Biochem. Journ.*, xxv, 1095.
HOPKINS, F. G. 1921. *Biochem. Journ.*, xv, 268.
1929. *Journ. Biol. Chem.*, lxxxiv, 269.
HUNTER, G. AND B. A. EAGLES. 1927. *Journ. Biol. Chem.*, lxxii, 167.
KENDALL, E. C., F. MCKENZIE AND H. L. MASON. 1929. *Journ. Biol. Chem.*, lxxxiv, 657.
MASON, H. L. 1930. *Journ. Biol. Chem.*, lxxxvi, 623.
Organic Syntheses, 1924, iv, 31; 1925, v, 63. John Wiley & Sons, New York.
PIRIE, N. W. 1930. *Biochem. Journ.*, xxiv, 51.
SCHELLING, V. 1932. *Journ. Biol. Chem.*, xcvi, 17.
SULLIVAN, M. X. 1926. *Pub. Health Rept. U. S. P. H.*, xli, 1030.
THOMPSON, J. W. AND C. VOEGTLIN. 1926. *Journ. Biol. Chem.*, lxx, 793.

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